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NEMATOLOGICA

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OBSERVATIONS ON HETERODERA CACTI FILIPJEV ET SCH. STEKHOVEN AND MELOIDOGYNE SPP. ON IMPORTED CACTUS PLANTS WITH A LIST OF NEW HOST RECORDS *)

BY

J. F. SOUTHEY

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In 1932 ADAM described *Heterodera* cysts found on Phyllocactus ackermanni and Cereus speciosus in Holland. In 1933 cysts were found at the same locality on Epiphyllum sp. (VAN POETEREN, 1934). From Adam's description, FILIPJEV and SCHUURMANS STEKHOVEN (1941) concluded that these cysts belonged to a new species and named it *Heterodera cacti*. Since that time there have been only a few records of the occurrence of this eelworm. It was again recorded in Holland on Phyllocactus sp. by OOSTENBRINK (1953). B. A. COOPER (*in litt.*, 1953) found *H. cacti* cysts on Dolichothele longimamma Britton & Rose in a glasshouse in Lincolnshire, England, and SOUTHEY (1954) reported their occurrence on a plant of Coryphantha sp. imported from Spain. Cysts of this species have also been intercepted with plants imported into the U.S.A. and Canada (HUNT, 1952, '53 & '54; BAKER, 1953).

T. GOODEY (1940) listed the following genera of Cactaceae as hosts of root-knot eelworm (*Meloidogyne* spp.): Cephalocereus, Cereus, Echinocactus (5 species), Echinopsis, Epiphyllum, Lobivia (2 species), Mammillaria (2 species), Opuntia, Rhipsalis. Most of these were from the records of BUHRER in the U.S.A. MAMELI CALVINO (1950) recorded Cereus pruinosus and C. serpentinus as hosts of root-knot eelworm in Italy. CHITWOOD (1949) refers to *Meloidogyne incognita* from Mexican cactus, but does not mention the species. GOFFART (1955) has recently reported *Heterodera cacti* and *Meloidogyne* spp. on cacti imported into Germany and states that these nematodes occur in various parts of that country.

In October, 1954, sample cactus plants from two nurseries in Eng-

*) Received for publication: May 15, 1956.

Most of the plants infested with *Meloidogyne* spp. showed typical galls on the roots. The galls were usually not more than about 5 mm. in diameter. However, one plant, (Lemaireocereus marginatus) showed a large swelling at the base of the stem, in the region of the hypocotyl. Below this swelling, which contained many females of *M. incognita* var. *acrita*, the root system had almost completely decayed (Fig. 1, F).

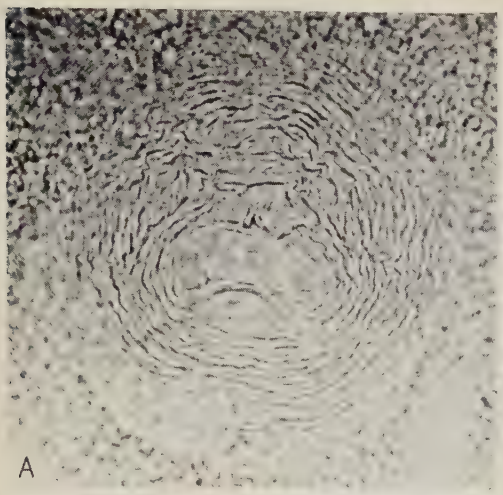
Morphology of H. cacti cysts

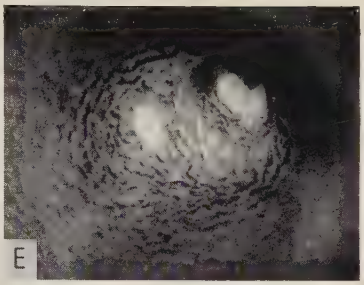
Examination of cysts of *H. cacti* from different populations showed that they agreed closely with ADAM's description (1932) and with the observations of OOSTENBRINK (1953). Fig. 2 shows a group of typical

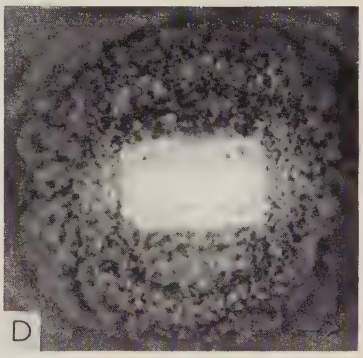
Fig. 2. A group of typical cysts from one population.

cysts from one population. They are easily recognized by their small size and rounded shape: it is quite common to find individual cysts in which the breadth is greater than the length, excluding the neck and vulval cone. The cysts are very variable in colour; when freshly formed they are golden yellow or pale orange, finally becoming dark brown. Only slight indications of a sub-crystalline layer were observed, in the form of a fine waxy deposit or 'bloom' on freshly formed cysts. This could be rubbed off, but could not be detached as a continuous crust as in some other *Heterodera* spp. No egg sac or other gelatinous secretion was observed.

The structure of the vulval cone is shown in Fig. 1, D & E. The fenestra of COOPER (1955) forms a roughly circular area crossed by the vulva, whose margins remain delicate and transparent and are







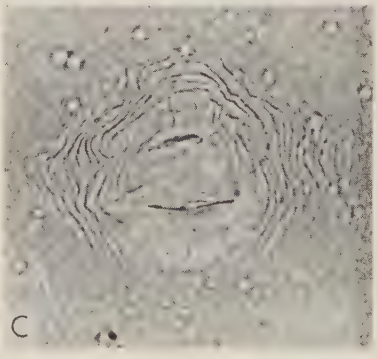


Fig. 1.

A-C. Perineal patterns of root-knot nematodes from cacti (all photographed to the same scale). A — *Meloidogyne incognita*; B — *M. incognita* var. *acrita*; C — *M. arenaria* (?). D. & E. Vulval cone of *Heterodera cacti*. D — End-on view of cone of yellow cyst showing vulva; E — Cone of old brown cyst displaced to one side, showing anus (to the left). F — Plant of *Lemaireocereus marginatus* with gall due to *M. incognita* var. *acrita* at base of stem.

not sclerotized to form a firm 'bridge' as in most other *Heterodera* spp. with lemon-shaped cysts, i.e., the cyst is circumfenestrate. In fresh cysts the semifenestrae on each side of the vulva may be partially or completely covered by granular opaque material which is easily detached (Fig. 1, D). In older cysts the vulval lips and adjacent tissue are soon broken away to leave a more or less circular hole. The anus is conspicuous and situated on one side of the vulval cone (Fig. 1, E).

Relationships

In the absence of a sclerotized vulval bridge, cysts of *H. cacti* resemble those of *H. weissi* and *H. rostochiensis,* all three species being circumfenestrate. *H. cacti* has several other features in common with *H. rostochiensis,* namely the absence of a conspicuous sub-crystalline layer, the absence of a gelatinous egg-sac, an intermediate yellow or golden stage in cyst-wall coloration and an almost spherical shape. In these features it differs from most other lemon-shaped species, but like them, and unlike *H. rostochiensis,* it has a prominent vulval cone; also, the vulval slit is long, extending almost the full width of the fenestra, and not short as in *H. rostochiensis.* Further work is needed on the host preferences of *H. cacti,* but so far it does not appear to have been found on any plants other than Cactaceae. If it is restricted to this family it seems likely that it originated from Central and South America, the natural home of these plants.

Practical Considerations

It is thought that *H. cacti* and *Meloidogyne* spp. should be regarded as important pests of cactus plants because of the damage which they do to the root systems. Control may be effected by cutting off roots or taking stem cuttings and rooting in a sterilized soil mixture or sand. If removal of roots is undesirable, hot-water treatment might be possible. One grower (GILBERT, 1955) has claimed good results in preliminary tests with this method.

I am much indebted to Mr. Gordon Rowley and Mr. A. Boarder for their help in naming many of the cactus plants. Messrs. R. C. Dale and D. Page discovered most of the infestations and sent me samples of the plants. Acknowledgements are also due to Mr. W. F. Buck who took the photographs.

ZUSAMMENFASSUNG

Das Vorkommen von *Heterodera cacti* Filipjev & Sch. Stekhoven und von *Meloidogyne*-Arten an nach England in den Jahren 1954 und 1955 eingeführten Kakteen wird beschrieben; eine Liste der Wirtspflanzen wird aufgeführt. Zwei Arten und eine Varietät von *Meloidogyne* wurden identifiziert, nämlich *M. arenaria, M. incognita* und *M. incognita* var. *acrita.* Es folgt eine weitere Beschreibung der Zysten von *H. cacti.* Die möglichen Verwandtschaften dieser Art werden diskutiert. Man vermutet, dass beide Nematoden als Kaktusschädlinge eine Gefahr darstellen, da sie grössere Wurzelschäden verursachen können. Das Einsetzen von wurzellosen Setzlingen in sterilisierten Boden oder Sand wird als Bekämpfungsmethode vorgeschlagen. Eine Warmwasserbehandlung dürfte auch möglich sein.

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(*Nematologica*, II (1957) : 7-15)

GROWTH, INVASION AND ROOT DIFFUSATE PRODUCTION IN TOMATO AND BLACK NIGHTSHADE INOCULATED WITH POTATO-ROOT EELWORM *)

BY

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In the summer of 1951 and again in 1953, attempts were made to compare the variations in growth, root diffusate production and extent of root invasion by the potato-root eelworm, *Heterodera rostochiensis*, Woll. in a favourable and an unfavourable host subjected to varying rates of nematode inoculation. The hosts chosen were tomato, *Lycopersicum esculentum*, Mill. and black nightshade, *Solanum nigrum*, L. Tomato is a satisfactory host and is known to produce an active root diffusate; potato-root eelworms will penetrate the roots and produce mature males and females. Black nightshade, on the other hand, is an unsatisfactory host in that invading larvae normally fail to mature beyond the third stage (Doncaster, 1953) but like tomato, it produces an active root diffusate (Russel, Todd & Waring, 1949).

METHODS

Seedlings of black nightshade and of tomato were planted in pots of an unsterilized 3:1 loam-sand mixture. For each species of plant, sixteen pots of this mixture were put up with no eelworm infestation (rate 0), sixteen were inoculated with cysts of the potato-root eelworm equivalent to 5 eggs and larvae per gram of soil (rate 1), sixteen with an inoculation equivalent to 30 eggs and larvae per gram of soil (rate 2) and sixteen with an equivalent of 180 eggs and larvae per gram of soil (rate 3). In each series of sixteen randomised pots four replicates were marked for treatment at the end of each of the four periods, one, two, four and eight weeks. A further set of sixteen pots of loam-sand mixture with no inoculation or plant was set aside as a control series for the root diffusate tests. All pots were sunk in a shady gravel plunge at the beginning of the experiment.

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The treatments at the end of each period consisted of leaching the appropriate pots with 50 ml. of tap water, the leachings being poured through each pot four times, collected and stored at a temperature of 4° C. The plants were then very carefully washed free from the soil, and fixed and stored in formal-acetic alcohol. Each sample of root diffusate was tested on duplicate batches of about 200 cysts obtained by weighing (Fenwick & Reid 1951), the hatched larvae being removed weekly for counting.

The plants were partially dried by gently pressing them between towels, the root systems were cut off at the crown and then weighed immediately. Following this they were stained in hot acid fuchsin lactophenol and the larvae within them were counted. In 1951 the counts were done on the stained larvae in the entire root systems which were spread out in a petri dish under the binocular microscope. However, the roots of many of the eight-week old plants inoculated at the light and the intermediate rates were so large that estimation of the larvae which they contained could not be attempted. In 1953, however, the plants from the 1951 experiment, in which the eelworms had been counted, were used in a test of the accuracy of the "Waring Blendor" technique for assessing eelworm numbers within roots (Taylor & Loegering, 1953, and Williams & Winslow, 1955). The stained complete roots were rinsed in acidified water, cut into short pieces with scissors and put into the "Waring Blendor" flask with about 60 ml. of acidified water. It was found that the eelworms suffered little damage even after five minutes treatment in the "Blendor" running at full speed, but in spite of this rather long processing a few nematodes still remained inside fragments of root. In order to minimise damage to the nematodes and at the same time to ensure that the majority were liberated, a time of three minutes was considered the most satisfactory.

Unlike the methods employed by Taylor & Loegering and by Williams & Winslow the suspension of nematodes and macerated root tissue was not sieved as it was found that some eelworms were lost on the coarse sieve through not having been liberated from the larger root fragments and an appreciable number of second stage larvae were lost through the fine sieve, which in this case was bolting silk of about 160 meshes to the inch. The complete suspensions were therefore made up to 100 ml. and eelworm counts from aliquot samples agreed very closely in every case with the counts made in 1951. In the 1953 repetition of the experiment, therefore, estimation of the

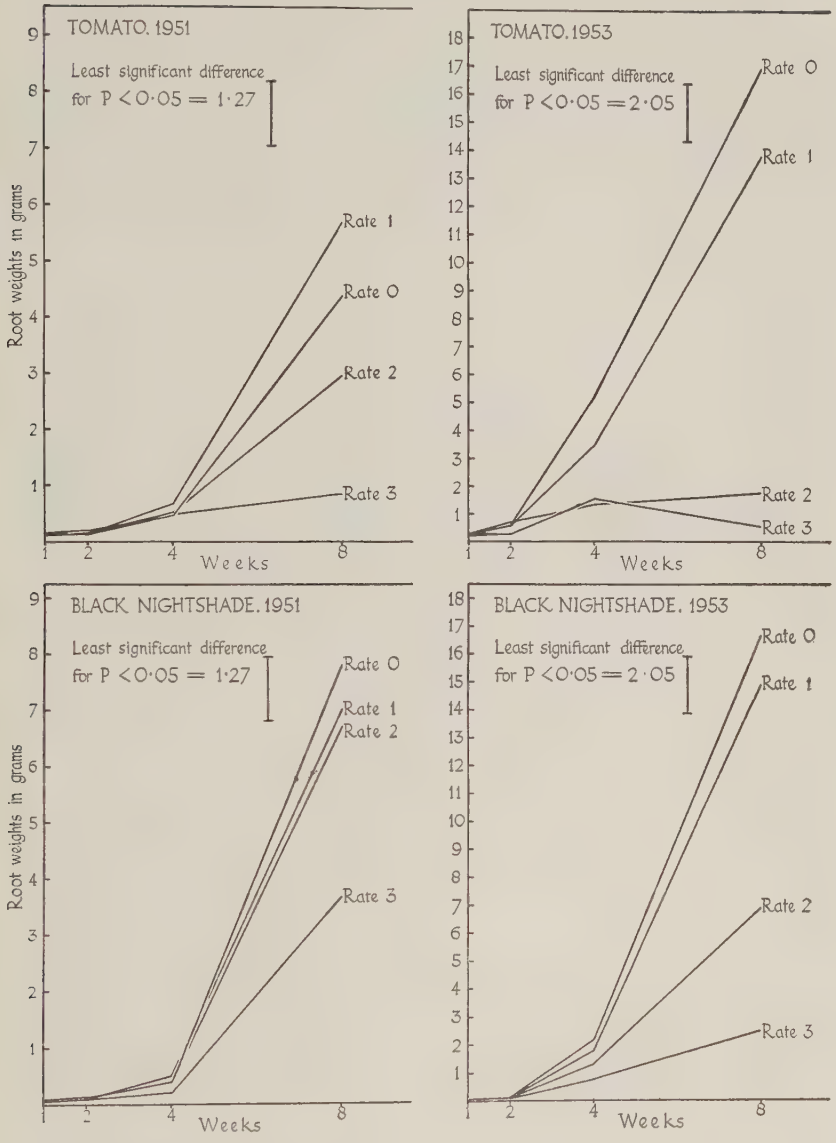

Fig. 1. The effect of inoculation on root development.

number of larvae within the host plant was made by the "Waring Blendor" technique. Where the root systems weighed as much as five grams the suspensions were washed into a measuring cylinder, made up to 100 ml. and aliquot samples were withdrawn for counting.

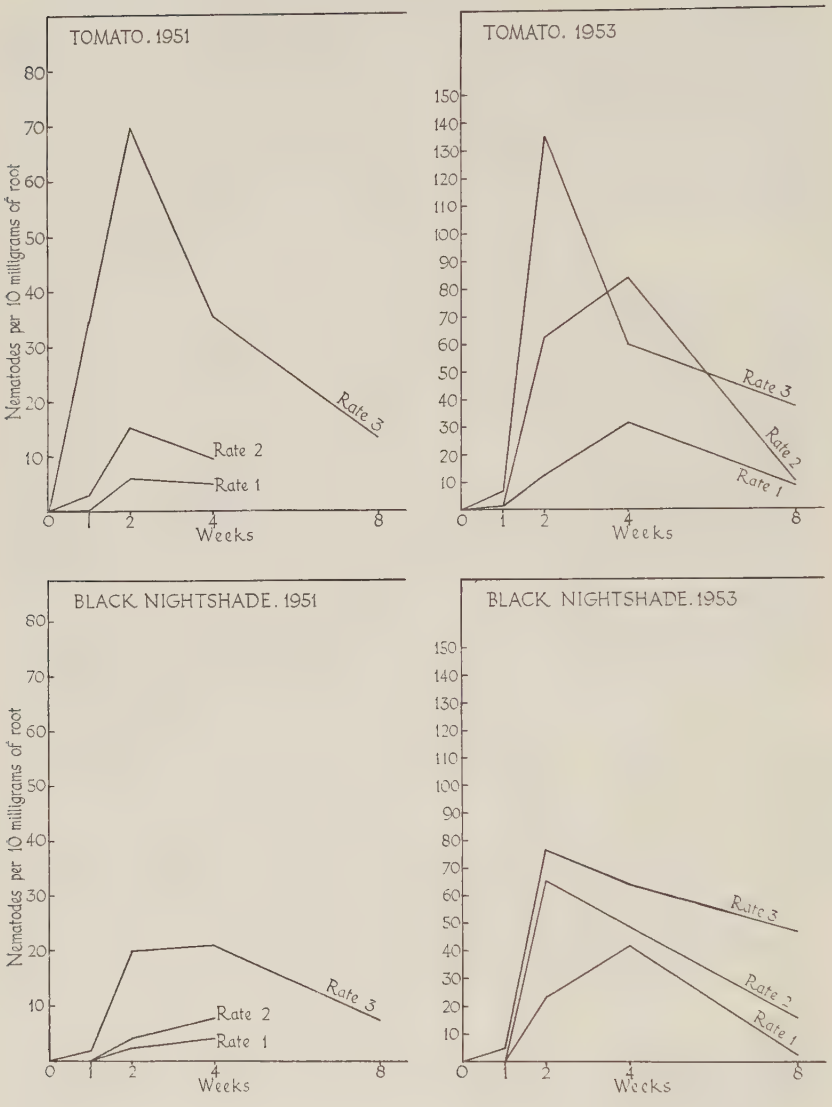

Fig. 2. Factors influencing the population density of *H. rostochiensis* in roots.

Where the root systems were smaller, however, the suspensions were centrifuged down to a convenient volume and aliquots of constant proportion were used for counting. In every case one tenth by volume of the root system was examined in this way.

RESULTS

In the experiment of 1951 the plants made noticeably poor growth, possibly due to the use of three-inch pots which were undoubtedly too small. Four-inch pots were therefore used in 1953 and development of the uninoculated plants was considered normal. The results of the experiments are presented in four figures.

Root development

Figure 1 shows root development at the four rates of inoculation. Analysis of variance on the data on root weights was carried out in arithmetic form and it is therefore possible to indicate by linear measurement on the figure the least significant differences between points. It is seen that in neither year was black nightshade as seriously affected by inoculation as tomato. In both years black nightshade showed significant effects of inoculation only by the eighth week and in 1951, when growth was generally poor, only the heavy rate of inoculation had any appreciable effect. In 1953 however, the intermediate rate was enough to cause noticeable stunting. At the end of the experiment all heavily inoculated nightshade and tomato plants appeared quite incapable of recovery and the tomato plants inoculated at the intermediate rate also reached a similar condition.

In 1953, when growing conditions were better, inoculation had a quicker and more pronounced effect on the tomato plants; stunting of the root systems due to the higher inoculation rates became apparent at four weeks and the light rate was sufficient to cause definite stunting by the eighth week.

Nematode populations within roots

Of the two host species, tomato was generally the more susceptible to invasion and bore not only the greater total number, but the greater density of parasites. Figure 2 shows that the maximum number of larvae in unit weight of root of each host species was more or less proportional to the inoculation rate, and in every case the peak lay between the second and fourth week.

If the numbers of nematodes in the entire root systems are considered, it is seen in figure 3 that whereas in nightshade the total number progressively increased, in tomato it fell off sharply between the fourth and the eighth week.

In 1953 records were kept of the numbers of potato-root eelworms

in the various life cycle stages which were found in the roots. From black nightshade a total number of 1013 larvae were counted and of these only 6 had reached the third stage. In the tomato plants a total of 1361 larvae were counted and of these 58 had reached stages between the fourth and the adult stage by the fourth week and a further 115 by the eighth week. There was a decline in the number of second and third stage larvae from 698 at the fourth week to 273 at the eighth.

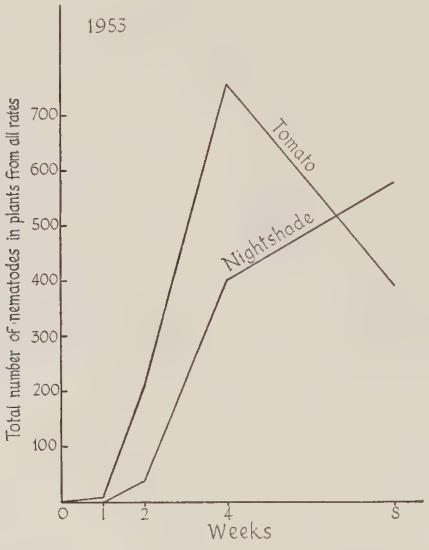

Fig. 3. Total numbers of *H. rostochiensis* in host plants.

Root diffusate production

As in the case of root development, root diffusate production appeared to be more affected by inoculation in 1953 than it was in 1951, when growing conditions were less favourable (fig. 4). In 1951 the tomatoes showed little reaction to inoculation until the eighth week when root diffusate production from the heavily inoculated plants was less than from the others. At the end of the first week's growth in this experiment, the tomatoes inoculated at the heavy rate gave a relatively high root diffusate output: it is possible that this was a genuine stimulatory effect, and perhaps comparable with it was the fact that at the second week in the 1953 experiment the lightly inoculated tomatoes were producing the most active root diffusate.

Under the improved growing conditions in 1953, tomato-root diffusate production was considerably affected by variations in inocula-

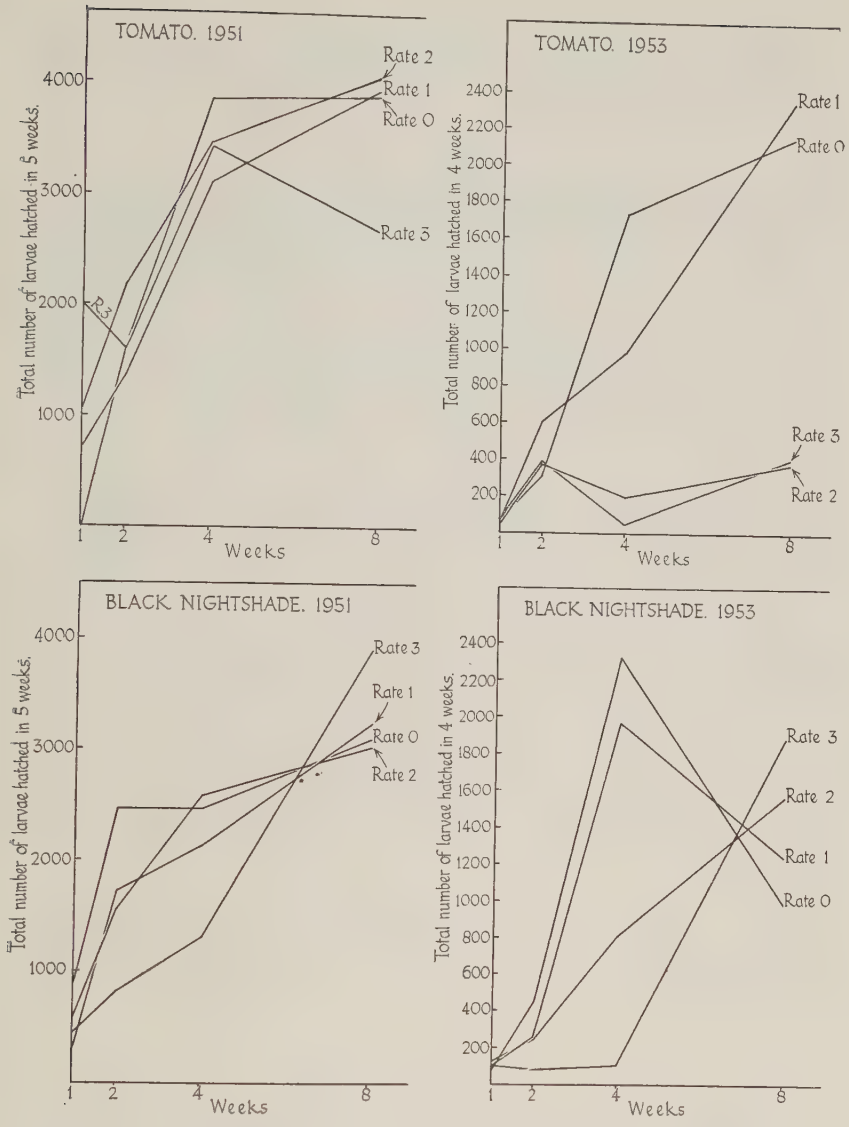

Fig. 4. The effect of inoculation on root diffusate production.

tion rate. There was a suggestion that the light inoculation rate caused some check between the second and fourth week and that there was a subsequent recovery. The intermediate and heavy rates caused a very severe check between the second and fourth week and this was followed by a slight but definite recovery.

(*Nematologica*, II (1957): 16-18)

ELECTRON MICROSCOPY OF GOLDEN NEMATODE CYST WALL *)

BY

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The most detailed account of the structure of the cyst wall of *Heterodera rostochiensis* is that of WIESER (1953) in which he described the layers of the cuticle in several species of *Heterodera*. Much of his evidence was obtained from light-microscope studies of sections 15 to 20 microns thick cut with a freezing microtome. The present paper is a description of the wall of the mature golden nematode cyst as it appears in electron micrographs of ultrathin cross sections. The terminology of WIESER will be used.

Cysts obtained from soil at the Golden Nematode Laboratory, Long Island, New York, were soaked in water for several days. The cysts were then cut open and the contents scraped out of those that contained eggs and living larvae. Pieces of the wall from these cysts were then fixed for 5 hours in a buffered 1 % solution of osmium tetroxide made by the method of PALADE (1952). They were washed in several changes of distilled water, dehydrated by an ethyl alcohol series, and embedded in a mixture of butyl and methyl methacrylate (NEWMAN *et al.*, 1949). Ultrathin sections were cut with glass knives on a thermal expansion single-pass microtome, and the sections were placed on collodion-coated grids for examination with the electron microscope. Freezing microtome sections 10 to 20 microns thick were cut of cyst wall that had not been fixed and also of cyst wall following fixation. These sections were examined with the light microscope.

Light-microscope examinations of the sections revealed a structure that in general appeared similar to that described by WIESER. The main division of the wall into two layers, the exocuticle and the endocuticle, was obvious, but the number of subdivisions within these

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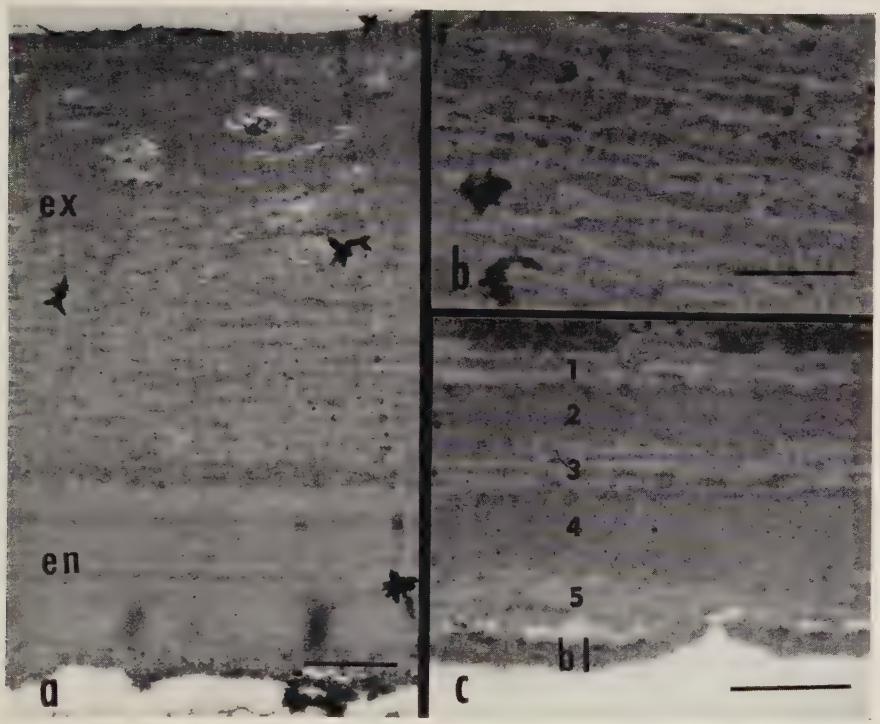

Fig. 1. a) Cross section of cyst wall showing exocuticle (ex) and endocuticle (en). Electron micrograph, × 12,000. b) Part of exocuticle showing irregular layers of two materials of different densities. Electron micrograph, × 16,000. c) Endocuticle, showing five distinct bands plus the basal lamella (bl). Electron micrograph, × 16,000. Bar in lower right of each picture equals one micron.

layers seemed less definite. No structural differences were observed between fixed and unfixed cyst wall. In sections of both fixed and unfixed material, there appeared to be faint radial striations in the exocuticle, which were assumed to be the fibrils described by WIESER.

In the electron micrographs we have always observed a division of the cyst wall into the two principal layers, the exocuticle and endocuticle (Fig. 1a). These layers are separated by a well-defined line of demarcation. The details, however, in both the exocuticle and endocuticle differ from those reported by WIESER. Within the exocuticle it is not possible to distinguish the various layers that he described. The exocuticle appears to be composed of one material interspersed with a second material of greater density to electron scattering. The materials are laid down in irregular layers oriented in a direction parallel to the edge of the cyst (Fig. 1b). The proportion of dense to less dense material is greater near the middle of the exocuticle than near the line of demarcation and the proportion increases as the outer edge is approached. The dense area (Fig. 1a) corresponds approximately to that said by WIESER to be occupied by the fiber and fibrillar layers; however, the electron microscope images no radially oriented structures that could be construed to be the fibrils suggested by light-microscope studies. Any apparent radial lines in the pictures can always be shown to be knife marks in the sections. A thin surface layer of a very dense material is present at the outer edge of the exocuticle. In many of the sections, only fragments of this material remained. This layer is too thin (approximately 0.03 microns) to be resolved by the light microscope.

A distinct banding is evident in the electron micrographs of the endocuticle. Fig. 1c shows the 5 bands that are evident. Another layer, which often appears to be separated from the wall itself, probably corresponds to the basal lamella mentioned by WIESER (Fig. 1c, bl). In micrographs that show the banding most clearly, the detail observed in bands 1, 3, and 5 of Fig. 1c appears similar. No structure has been observed in the other two bands (bands 2 and 4 of Fig. 1c).

Thus the two main divisions of the cyst wall that can be distinguished with the light microscope also appear in the electron micrographs of ultrathin sections. Whereas more detail can be seen in the endocuticle than has been described from light microscope studies, the details of the exocuticle that have been reported from the studies with the light microscope are absent in the electron micrographs. Perhaps the most conspicuously absent morphological structure is the fibrillar

layer that was reported by WIESER to be especially well developed in *H. rostochiensis*. It should be pointed out that contrast in the image formation in the electron microscope is caused by differences in density to electron scattering. These differences may or may not correspond to those factors that produce contrast in the light microscope. The electron microscope, however, gives much higher resolution than the light microscope, and it is improbable that a fibrillar layer, if such existed, would not be imaged in the electron microscope. No explanation can be made at present of the appearance in the light microscope of radial striations in the exocuticle, but the assumption that the striations represent radially-oriented fibrils must be questioned on the basis of the evidence from these electron microscope studies.

SUMMARY

An exocuticle and an endocuticle are visible in electron micrographs of ultrathin sections of golden nematode cyst wall. More detail can be seen in the endocuticle than has been reported from light microscope studies. Details of the exocuticle described from light microscope observations, are not imaged in the electron microscope.

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TWO NEW NEMATODES FOUND ASSOCIATED WITH SOY-BEAN ROOTS *)

BY

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Carcharolaimus formosus n.sp.

Nematodes of the genus *Carcharolaimus* Thorne, 1939, are known to occur in Brazil only at Santa Cruz do Sul (Rio Grande do Sul) and at Escola Superior de Agricultura "Luiz de Queiroz", in Piracicaba (State of S. Paulo). In the last place, the individuals have never been numerous, only one or two specimens being obtained from each of several soil samples.

The only species already recorded for the brazilian fauna (*C. pizai* Lordello, 1953) is now known to be a predacious form, feeding on small oligochaetes, the setae of which being easily observed within the intestinal lumen. Such behavior is possibly displayed by the species here described as *C. formosus* n. sp.

Body practically cylindrical, only slightly tapering to extremities. Cuticle faintly transversely striated. Lips prominent, broadly expanded, somewhat angular, set off from neck by a rather deep constriction, provided with two circlets of conspicuous papillae. Vestibular cuticularized structure denticulated, the largest denticles located on the base of the structure. Amphids stirrup-shaped, very small, one-fourth as wide as neck. Spear strongly built; guiding-ring single, heavily cuticularized, provided with supporting ribs. An ovoid body, of unknown nature is present in front of guiding ring. Oesophagus first a slender tube, which expands to form a broad cylindrical basal portion, its lining faintly plicated transversely. Cardia roughly conical and short; intestinal cells filled with coarse granules. Pre-rectum indiscernible; anus located in a slight depression, provided with strongly developed dilatator muscles. The reason for such a powerful muscular provision

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may be found in the physical nature of the faeces, which must include structures of the victims that were not digested.

A peculiar feature of *C. formosus* n. sp. is found in the conspicuous pores present along the ventral and lateral fields; these pores are

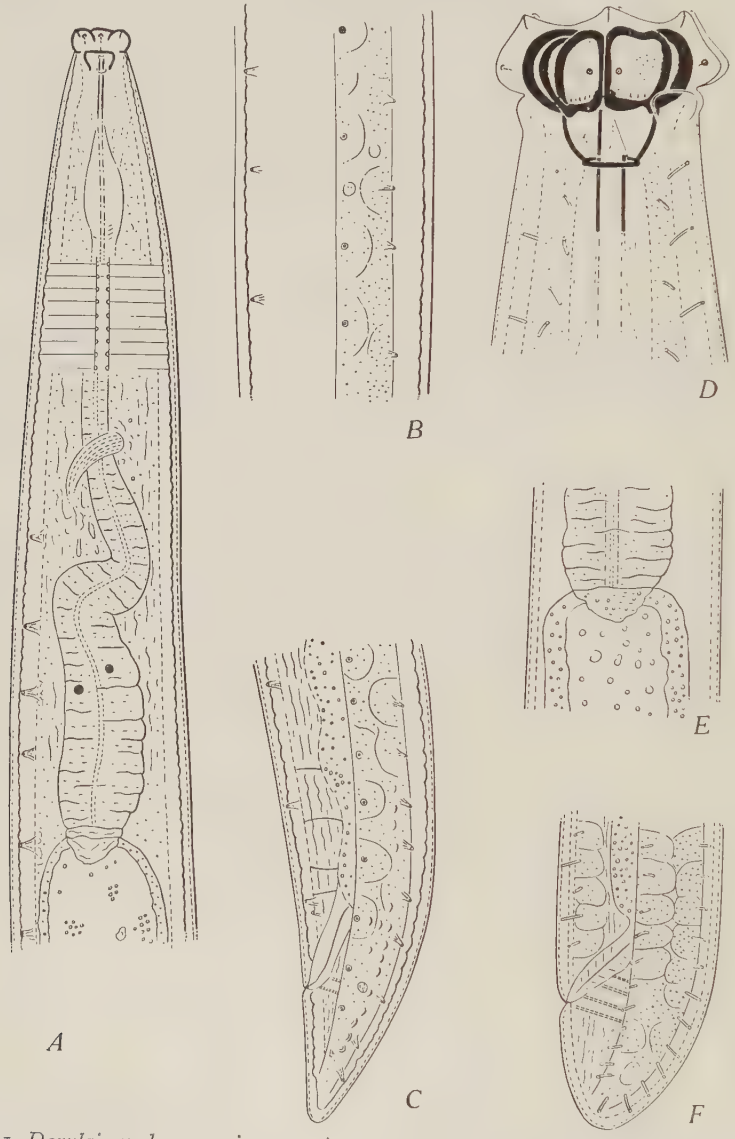

Fig. 1. *Dorylaimus bauruensis* n. sp. A — anterior end of body; B — lateral field and ventral pores; C — posterior portion of body. *Carcharolaimus formosus* n. sp.: D — head end; E — region of cardia; F — posterior end of body.

connected with large glandular cells of the longitudinal chords through rather long canals. From figs. 1, D and 1, F, it can be seen that there are two series of pores on each of the referred longitudinal fields. At the level off the vulva, the lateral fields are 14.0 microns wide, occupying slightly less than one-fourth of the body width.

Vulva transverse; ovaries two, reflexed, the anterior ovary a little more developed than the posterior (9.8 and 5.9% of total length, respectively).

Tail short, subconoid; no lateral papillae have been observed.

Diagnosis — Only two *Carcharolaimus* species provided with denticulated labial structure are known: *C. dentatus* Thorne, 1939, and *C. pizai* Lordello, 1953. From *C. dentatus, C. formosus* differs in: a) posterior position of vulva (V = 48.0%: V = 57.4%); b) lips visibly more expanded, being as broadly expanded as in *Discolaimus* species; c) presence of pores along the ventral and lateral fields, which are connected with large cells of the chords through rather long canals; and, d) absence of glandular bodies at base of oesophagus.

From *C. pizai, C. formosus* differs in: a) labial region much more broadly expanded; b) presence of the described pores; and, c) longer and slenderer body (total length = 2,337.0 : 1,635.0 microns; width = 50.0 : 65.0 microns).

Male and food habits unknown.

Measurements (in microns) — Total length: 2,377.0; width: 50.0; spear: 18.5; spear aperture: 11.6; labial diameter: 29.0; oesophagus: 582.6; basal portion of oesophagus: 418.0 × 25.0; rectum: 30.0; tail: 29.0; a = 46.7; b = 4.0; c = 80.5; V = 57.4%.

Type locality — Soil around roots of soybean (*Glycine max* (L.) Merrill) collected at Bauru, State of S. Paulo; the roots were decaying due to invasions by root-knot nematodes (*Meloidogyne* spp.).

Key to species of *Carcharolaimus* Thorne, 1939

1. Cuticularized labial structure not dentate 2
   Cuticularized labial structure dentate 3
2. Neck short, b = 5 . . . . . *C. teres* Thorne, 1939
   Neck longer, b = 3.7 . . . *C. rotundicauda* (de Man, 1880) Thorne, 1939
3. Vulva in front of middle of body (V = 48%), glandular bodies at base of oesophagus . . . . . *C. dentatus* Thorne, 1939
   Vulva behind middle of body (V = 56.2-57.4%), no glandular bodies at base of oesophagus 4
4. Larger and slenderer species (length = 2,337.0 microns, width = 50.0 microns), lips broadly expanded . . . . . *C. formosus* n. sp.
   Shorter and wider species (length = 1,635.0 microns, width = 65.0 microns), lips not expanded .. . . . . *C. pizai* Lordello, 1953

Dorylaimus bauruensis n. sp.

D. bauruensis n. sp. is doubtless the most interesting *Dorylaimus* recorded for the brazilian fauna. Actually, several anatomical features make this species very outstanding, as can be seen from the following description.

Body slightly tapering anteriorly and posteriorly to a conical and somewhat arcuated tail. Cuticle smooth; subcuticle characteristically undulated; the top line of each undulation appears as a transversal line crossing body in a lateral view. However, such transverse lines (only in part represented in fig. 1, A) are visible solely from region of spear to the anterior extremity of the basal portion of oesophagus; from that region to tail, the lines gradually disappear.

Lips distinct, round, set off from neck by a marked constriction. The cephalic papillae could not be studied accurately; but, two circlets of inconspicuous papillae seem to be present. Amphids stirrup-shaped, about one-half as wide as neck.

Spear weak, with double guiding ring. Two large nuclei of glands are seen in the basal portion of oesophagus. A flattened discoid formation is located between this basal portion and conoid cardia. Intestinal cells short, broad, round or somewhat polygonal, crowded with dark granules; from 6 to 7 cells make a circumference of the intestine. From level of vulva to anus, the intestine is undulated and it may be seen that there are suspensory bridges from the body wall to the intestinal wall. Anus provided with few but rather strong dilatator muscular fibers. Pre-rectum indiscernible, being possibly absent. The dorsal portion of intestine overlaps the rectum at the junction of these two parts of the digestive apparatus.

Sexual apparatus amphidelphic. Vulva transverse; vagina apparently provided with circular muscles, extending across body a little less than one-half of its width. Ovaries reflexed, obscure; anterior ovary more developed than posterior (14.6 and 10.6%, respectively, of total length). No eggs seen in the uterus; at anterior ovary, the largest oocyte, which would certainly be soon moved into uterus, measured only 17.0 × 13.3 microns.

Lateral fields rather narrow (13.3 microns at level of vulva), less than one-third as wide as body. Body muscles distinctly undulated.

The outstanding feature of the species is given by series of pores located in elevations, which are easily seen along body. Such structures, in a lateral view of body, are seen from region of spear to end of

tail, but their aspect varies according to the region under observation. There are lateral and ventral pores, distributed respectively along the lateral and ventral fields, and connected with rather large glandular cells of the longitudinal chords. The lateral pores are first rather close together (fig. 1, A); however, from the anterior portion of the oesophageal basal region, the fields become wider and the pores obviously are not so close together; at last, the distribution is that shown in fig. 1, B. On the posterior portion of body, besides the pores, scalelike structures are also present (fig. 1, C). Lateral papillae were not seen.

Along the ventral fields, the distribution of pores is similar; ventral pores only could not be observed in front of the level of the nerve ring.

Such an organization in the lateral and ventral chords suggests a peculiar mode of life which, unfortunately, is completely unknown.

Diagnosis — In the literature, no species resembling *D. bauruensis* could be found, with which a comparison should be made. The peculiarities presented by the species make it unique within the genus.

Male and food habits unknown.

Measurements (in microns) — Total length: 782.0; width: 46.5; spear: 21.6; spear aperture: 5.0; labial diameter: 13.3; oesophagus: 197.5; basal portion of oesophagus: 69.7 × 20.0; rectum: 26.5; tail: 34.8; a = 16.8; b = 3.9; c = 22.4; V = 46.7 %.

Type locality — A single female obtained from soil samples collected at Bauru, State of S. Paulo, around roots of soybean heavily disfigured by root-knot nematodes.

The type specimens of the two new species described in this paper are in the writer's collection.

RESUMO

Faz-se, neste trabalho, a descrição de dois novos nematódeos de vida livre no solo, coletados em Bauru, Estado de Sao Paulo, Brasil, ao redor de raizes de soja.

1) *Carcharolaimus formosus* sp. n. é a segunda espécie do gênero referida para a fauna brasileira pois, em 1953, foi publicada a descrição de *C. pizai* Lordello, procedente de Piracicaba, Estado de S. Paulo.

É provável que *C. formosus* se alimente de oligoquetas, tal como *C. pizai*, em cujo intestino podem ser vistas cerdas e outras estruturas pertinentes àqueles anelídeos, que não foram digeridas.

Apenas se conheciam duas espécies de *Carcharolaimus*, cuja complexa estrutura labial é provida de dentículos, a saber: *C. dentatus* Thorne, 1939, e *C. pizai* Lordello, 1953.

De *C. dentatus, C. formosus* difere pela posição posterior da vulva (V = 48% : V = 57,4%); pelos lábios visìvelmente mais expandidos, tão desenvolvidos como em *Discolaimus*; pela presença de poros ao longo dos campos laterais e ventral, os quais se acham em conexão com grandes células das cordas longitudinais atravez de canais relativamente longos; e, pela ausência de corpos glandulares na base do esôfago.

De *C. pizai, C. formosus* difere pela região labial muito mais expandida; pela presença dos poros referidos; e, por possuir corpo mais longo e esguio (comprimento total = 2.337,0 : 1.635,0 micros; largura = 50,0 : 65,0 micros).

É organizada uma chave para auxiliar o reconhecimento de tôdas as espécies conhecidas do gênero *Carcharolaimus* Thorne, 1939.

2) *Dorylaimus bauruensis* sp. n. é, sem dúvida, o *Dorylaimus* mais interessante até o presente referido para a fauna brasileira. O seu notável característico é dado por séries de poros localizados em elevações, fàcilmente visíveis ao longo do corpo. Tais poros relacionam-se com grandes células glandulares das cordas longitudinais. Na porção posterior do corpo, além dos poros, aparecem estruturas que recordam escamas. Tal organização sugere um modo de vida peculiar, o qual, infelizmente, é completamente desconhecido.

Compulsando a literatura, nenhuma espécie de *Dorylaimus* foi encontrada, que se assemelhasse a *D. bauruensis*. Constitui uma forma única dentro do gênero.

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(*Nematologica*, II (1957): 25-31)

PARASITISM AND PATHOGENICITY OF XIPHINEMA DIVERSICAUDATUM, AN ECTOPARASITIC NEMATODE [1], [2]

BY

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From a survey of commercial rose-growing greenhouses (SCHINDLER, 1956), it was ascertained that 2 genera of nematodes, *Xiphinema* (dagger nematodes) and *Pratylenchus* (meadow nematodes) (BUHRER, 1954), were the most wide-spread and numerous of the suspected plant parasitic forms consistently found to be associated with roses exhibiting poor growth and chlorosis.

Because of the frequency with which *Xiphinema diversicaudatum* (Micoletzky, 1927) Thorne, 1939 was found associated with reduced vigor, chlorosis, and galled roots on roses, and because parasitism or pathogenicity of *Xiphinema* spp. was unproven, it was decided to conduct greenhouse experiments with this nematode in an attempt to discover its parasitic relationships and its pathologic effects.

Although *Xiphinema* has often been mentioned as a possible plant parasite since COBB (1914) first suggested this, no experimental proof has been given. STEINER (1949, 1952) and CHRISTIE (1952 b) considered *Xiphinema* spp. as probable plant parasites while the latter considered *X. americanum* Cobb, 1913 one of the four nematode pests of major importance in the southeastern United States. Both workers considered this species a serious parasite of biennial or perennial trees and shrubs (CHRISTIE, 1952a, 1952b and STEINER, 1952). Several workers have shown convincing circumstantial evidence indicating the

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probable pathogenic role of the dagger nematode. THORNE and ALLEN (1950) examined samples of roots and surrounding soil from fig trees near Planada, California, which were affected with a condition known locally as "leaf drop". Numbers of *Paratylenchus hamatus* Thorne and Allen, 1950, and *X. index* Thorne and Allen, 1950, were found. The authors suggested that *P. hamatus* was a contributing, if not the primary, factor in this fig tree decline, with the possibility that *X. index* was also involved. CHRISTIE (1952a) consistently found *X. americanum* associated with a type of injury common to laurel oak, Quercus laurifolia, in central Florida. Roots of affected trees show extensive superficial necrosis and are largely devoid of small rootlets except for occasional clusters of short, stubby, branch roots. In 1955 ADAMS reported on a soil fumigation experiment in which young peach and apple trees growing in soil treated with benzene hexachloride showed increased growth, apparently due to the demonstrated control of *Xiphinema* sp.

MATERIALS AND METHODS

In an attempt to build up a population of *X. diversicaudatum* for future experimental use, soil samples from a commercial rose-growing greenhouse in Indiana were processed by washing and sieving through 30- and 100-mesh screens. The nematodes were collected from the 100-mesh screen and 3 groups of 200 specimens each were picked from a Syracuse dish. These were kept in watch glasses containing 1 to 3 ml. of water. Rooted cuttings of rose, variety Better Times, were planted in steam-sterilized soil in 10-inch clay pots and the 200 specimens of *Xiphinema* were pippetted onto the roots of the cuttings at the time of potting. Pots were then placed in a greenhouse where night temperatures of between 70° and 75° F. and day temperatures between 75° and 80° were ordinarily maintained.

When the soil of each pot was examined for nematodes, it was divided into several 2 to 3 lb. samples. Each sample was washed twice in a bucket of water and screened through 20-mesh and 100-mesh sieves. The nematodes were collected from the 100-mesh screen and dilution counts were made.

To demonstrate the cause or causes of the root galling almost invariably found on rose plants growing in *Xiphinema* infested soil, several greenhouse experiments were initiated. In all instances, the potting medium was either sterile or steam-sterilized to eliminate the possibility of other nematodes being involved. The inoculation pro-

cedure consisted of applying nematodes around the roots of the plant at the time of potting. Where specific numbers are given, the specimens of *X. diversicaudatum* were picked out by hand as described. Where approximate numbers are given, they were determined by making counts on aliquots of nematode inoculum. The upper $^1/_3$ to $^1/_2$ of the water from which the nematodes had settled was decanted after standing for at least 1 hour. This supernatant liquid, containing no nematodes but containing other biological or chemical factors which may have been associated with or secreted by the nematodes, was applied to the roots of plants as a treatment at the time of potting.

All experiments consisted of three or four treatments, depending on whether more than one concentration of *Xiphinema* inoculum was used. Treatments were as follows: 1) control, 2) supernatant water, 3) low number of nematodes, and 4) high number of nematodes. When treatments were replicated, the tests were designed as randomized blocks.

RESULTS AND DISCUSSION

Thirty-three months after inoculation, soil from one of the potted roses inoculated with 200 nematodes was examined. It was estimated that 17,000 *X. diversicaudatum* specimens were recovered after processing, a ratio of increase of 85: 1. Thirty-nine months after inoculation, two more pots were examined. These yielded 22,000 and 27,000 nematodes, a ratio of increase of 110: 1 and 135: 1, respectively. All the rose plants had poor root systems coupled with severe galling.

Since it can be assumed that a part of the nematodes are lost in the process of screening from the soil and also that pots in a greenhouse are not the most favorable environment for either the nematodes or the host plants, these ratios of increase were probably lower than might be obtained under the more favorable conditions in commercial greenhouse beds.

Parasitism of *X. diversicaudatum* was established by direct observation (Figure 1). Soybeans, Glycine max, variety Banzei, were grown in *Xiphinema* infested soil and after several weeks the roots were carefully washed free of soil and examined. Several specimens of *X. diversicaudatum* were found attached to the roots of the seedlings. They were observed for several days during which they remained attached and apparently feeding on the roots.

In a preliminary test to determine whether *X. diversicaudatum*

induced root gall formation (SCHINDLER, 1954), a rooted rose cutting, variety Better Times, was inoculated with 945 nematodes and planted in a 5-inch pot. Two control cuttings were planted at this time. The 3 plants were examined after 5 months and numerous galls were observed on the roots of the inoculated plant (Figure 2). Galling was characteristically an enlargement of the tip and a curling of the end of the root with apparent necrosis and shrivelling of the proximal portion. This response, designated as "curly-tip", appears to be typical and may be recognized with experience. Galls caused by dagger nematodes are somewhat similar to those caused by root-knot nematodes but differ in that they almost invariably involve the distal portion of the root. They were mistaken for root-knot galls when first seen on rose roots from commercial greenhouses. No galls were found on the roots of the control plants. It was estimated that 4,200 specimens of *X. diversicaudatum* were recovered from the soil of the inoculated pot while none were found in the soil from the control pots.

In two experiments, each replicated 4 times, Vermiculite was used as the potting medium in an attempt to obtain, for cytological work, galled and ungalled roots in a cleaner condition. Cuttings of clones of Manetti rose, of Rosa bracteata, and of variety Better Times were used. Inoculum consisted of 150 nematodes per plant in 1 experiment and 100 nematodes per plant in the other.

The experiments were terminated after 33 and 42 days. Only dead *Xiphinema* specimens were recovered from the inoculated pots and slight galling was observed on the roots of only 1 plant. Apparently Vermiculite is an unsuitable substitute for soil in experiments with this nematode genus and its use should be avoided.

An experiment using 3 rose plants, variety Better Times, in each of 4 treatments was conducted to determine whether *X. diversicaudatum* actually caused root galling. Five-inch pots with a mixture of 1 part of peat moss to 3 parts of sterilized soil were used. Fifty and 200 nematodes were used on each plant in treatments 3 and 4 respectively. After 38 days the roots of all plants were examined and those in the control and supernatant water treatments had no galls. Where 50 nematodes were used as inoculum the roots of each plant had several small to medium-size galls. Treatment 4, which consisted of 200 nematodes, caused several small to medium-size galls on one plant. The 2 remaining plants had roots with numerous small and medium-size galls in addition to several large galls.

Galling of roots of peanut, Arachis hypogaea, variety Virginia

Jumbo, by *X. diversicaudatum* was demonstrated by inoculating one of 3 seedlings with 200 specimens. After 45 days the roots of the plants were examined. Control plants were free of galls while the inoculated plant had the typical swollen and "curly-tip" roots (Figure 3). Galls were particularly evident near the crown of the plant, indicating that the later growth of the roots was rapid enough to overcome the effects of attacks by this number of nematodes.

The results obtained from the experiments described demonstrate that *X. diversicaudatum* causes a galling of roots of the host plants tested.

To determine pathogenic effects other than galling, rooted Manetti rose cuttings were given the 4 basic treatments listed with 4 replications. After inoculation with an estimated 500 and 10,000 *X. diversicaudatum* specimens per plant in treatments 3 and 4, plants were grown in 5-inch pots in the greenhouse. Within one month, an effect on growth of the plants was discernible. The experiment was terminated in 109 days, when plants in the various treatments showed marked growth differences. When the roots were washed free of soil, differences in root development were also observed (Figures 4 and 5). In all instances, in terms of the increase in length of the shoot or shoots and of the total plant weight (Table 1), the means in treatment 4 were significantly different from those of all other treatments. Plants in treatment 3 were significantly below the control plants in both shoot length and plant weight. There was no significant difference between treatments 2 and 3. With respect to increase in shoot length, the control plants were significantly better than plants in the supernatant water treatment, while they closely approached significance when measured in terms of plant weight.

A smaller experiment with rooted cuttings of a fig tree, *Ficus* sp., was designed. There were 2 replications of 3 treatments. Plants in treatment 3 were inoculated with approximately 2,600 nematodes. Sixty-three days after inoculation the plants showed large differences in amounts of new growth (Figure 6). Examination of the entire plant showed a severe stunting of the root systems of the plants in treatment 3 (Figure 7). There was a statistically significant increase in shoot length of control plants versus plants in other treatments (Table 1). Differences in mean plant weights were not quite large enough to be significant at the 5% level. Roots of plants in the treatments with *X. diversicaudatum* were so poor as to be practically absent (Figures 4, 5, 7) or were galled.

TABLE I

Growth effects of *Xiphinema diversicaudatum* (Micoletzky, 1927) Thorne 1939 on Manetti rose and Ficus sp.

Treatment		Rose (4 replications) Mean increase in shoot length (mm.)	Rose (4 replications) Mean plant wgt. (gm.)	Fig (2 replications) Mean increase in shoot length (mm.)	Fig (2 replications) Mean plant wgt. (gm.)
(1) Control		93.3	44.5	153	88
(2) Supernatant water		54.3	31.0	38	37
(3) Low nematode concentration		38.5	22.5	18	5
(4) High nematode concentration [a]		0	1.3	—	—
L. S. D. (for treatments 1, 2, and 3)	.05	34.5	14.6	98.1	Not significant at 5% level.
	.01	52.3	22.1	226.3	

[a] To avoid heterogenity of error variance treatment 4 was omitted from the analysis of variance since all values were zero for length and very small for weight.

In both of the experiments designed to determine effects other than galling, no parasitic nematodes were found in the soil from pots in treatment 2.

It can therefore be concluded that *X. diversicaudatum* is a plant parasite and is pathogenic in that it causes abnormal galling of the root tissues and inhibits the growth of the host plants. Since infestations of *X. diversicaudatum* were consistently found associated with roses exhibiting poor growth and chlorosis and since the pathological effects of this nematode on host plants have been demonstrated it can be concluded that *X. diversicaudatum* is a serious problem in greenhouse rose production.

Data also indicate the presence of an unknown factor, either biological or chemical, in the supernatant water. Effects of this factor were similar to those of the lower concentrations of nematodes on the growth of the plants, though no specimens of *Xiphinema* were recovered from any of the pots to which this supernatant had been added. These effects may have been due to the actions of fungi, bacteria, or viruses, alone or in combination; or they may have been due to the action of a chemical growth inhibitor secreted by the nematodes while in the supernatant water.

Fig. 1. *Xiphinema diversicaudatum* attached to, and feeding on, root of soybean, variety Banzei. (Approx. 15 ×).

Fig. 2. Portions of rose roots, variety Better Times, showing typical galls caused by *Xiphinema diversicaudatum*. (Approx. 2 ×).

Fig. 3. Galled roots of peanut, variety Virginia Jumbo, inoculated with 200 specimens of *Xiphinema diversicaudatum*; 45 days after treatment. (Approximately 4×).

Fig. 4. Growth inhibition of Manetti rose parasitized by *Xiphinema diversicaudatum*, 109 days after treatment: 1. control; 2. supernatant water; 3. approx. 500 nematodes; 4. approx. 10,000 nematodes.

Fig. 5. Four replications of a treatment in which each Manetti rose plant was inoculated with approx 10,000 *Xiphinema diversicaudatum* specimens, 109 days after treatment.

Fig. 6. Growth inhibition of Fig plants when parasitized by *Xiphinema diversicaudatum*, 63 days after treatment: 1. control; 2. supernatant water; 3. approx. 2,600 nematodes.

Fig. 7. Roots of Fig plants parasitized by *Xiphinema diversicaudatum*, 63 days after treatment: 1. control; 2. supernatant water; 3. approx. 2,600 nematodes.

ZUSAMMENFASSUNG

Xiphinema diversicaudatum, ein verdächtiger pflanzenparasitischer Nematode, trat in Gewächshäusern in Verbindung mit kümmernden Rosen auf. Um nachzuweisen, ob dieser Nematode parasitisch lebt und pathogen ist, wurden Kulturen angesetzt und mit diesen Manetti Rose, Rosa sp. (Varietät Better Times), Erdnuss (*Arachis hypogaea*, Varietät Virginia Jumbo), Sojabohne (Glycine max, Varietät Banzei) und Feige (*Ficus* sp.) infiziert.

Parasitismus wurde durch unmittelbare Beobachtungen an *X. diversicaudatum* nachgewiesen, der an den Wurzeln von Sojabohnen sog. Die Pathogenität ergab sich durch die Bildung von Wurzelgallen an verschiedenen Wirtspflanzen und durch die deutliche Wachstumsverzögerung bei Manetti-Rose und Feige, wenn sie mit Proben von *X. diversicaudatum* infiziert worden waren. Wegen ihrer Wirkung auf die Wirtspflanzen und ihres Vorherrschens in Rosen-Anzuchthäusern scheint der Nematode ein ernster Rosenschädlung zu sein.

Ein unbekannter biologischer oder chemischer Faktor, der das Wachstum der Rosen und Feigen verzögerte, schien in Wasser anwesend zu sein, das vorher grosse Mengen von *X. diversicaudatum* enthielt.

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A REVIEW OF THE NEMATODE GENUS TRICHODORUS WITH DESCRIPTIONS OF TEN NEW SPECIES [1])

by

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The nematode genus *Trichodorus* was established by COBB in 1913 when he described the species *Trichodorus obtusus*. Subsequently MICOLETZKY (1922) transferred *Dorylaimus primitivus* de Man 1880 to the genus *Trichodorus* and made *T. obtusus* a synonym of *T. primitivus*. THORNE (1939) illustrated and described specimens from Arlington Farm, Virginia, the type locality of *T. obtusus*, as representing *T. primitivus*. He recognized that these specimens differed from *T. obtusus* = *T. primitivus* of COBB 1913 and from *T. primitivus* of DE MAN 1880 but suggested that the differences were of the type that might be due to geographical variation. Specimens of *T. primitivus* collected in the Netherlands in 1952 were compared with the specimens collected by THORNE in 1931 from Arlington Farm and it was found these nematodes are not the same species because of the difference in the position of the supplementary papillae on the male tails as well as other morphological features. Since *T. primitivus* and *T. obtusus* of COBB 1913 were illustrated showing an identical arrangement of the papillae on the male tail it is concluded that the material collected by THORNE from Arlington Farm is not COBB's *T. obtusus*.

Prior to December 1955 it appeared unlikely that *T. obtusus* Cobb 1913 could be a synonym of *T. primitivus* since there was no record of the occurrence of *T. primitivus* in the United States other than the fact that MICOLETZKY (1922) and THORNE (1939) had indicated that *T. obtusus* was a synonym of *T. primitivus*. However, in December 1955 Dr. W. R. JENKINS collected specimens which proved to be *T. primitivus* from soil around the roots of mimosa on the eastern shore of Maryland in Talbot County. On the basis of this record I

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assume that it is possible that Dr. Cobb could have described *T. obtusus* from specimens of *T. primitivus* collected in Virginia. In addition, *T. primitivus* was collected from soil around the roots of boxwood in June 1954 by C. N. Keyser from an experimental plot located near Philadelphia, Pennsylvania. I have, therefore, concluded that Micoletzky was correct in placing *T. obtusus* in synonymy with *T. primitivus* and that the specimens collected by Thorne in 1931 from the type locality of *T. obtusus* represent a previously undescribed species.

Nematodes belonging to the genus *Trichodorus* have received considerable attention as ectoparasites of the roots of plants since Christie and Perry (1951) reported a species of *Trichodorus* to be the causative organism of stubby-root disease of certain crop plants in Florida. However, because the species investigated by these authors was not described or named, there has been considerable uncertainty as to the identity of the nematode species causing the stubby-root disease and its relationship to other species in the genus. The present study was undertaken in the hope that certain of the difficulties involved in the identification of *Trichodorus* species could be resolved. I am particularly indebted to Dr. D. J. Raski for collecting many of the specimens studied, to Dr. J. R. Christie and Mr. Vernon Perry for supplying numerous specimens of the species causing stubby-root disease and to Mr. Gerald Thorne for supplying specimens from Arlington Farm, Virginia. Mr. Armand Maggenti provided valuable assistance in measuring nematode specimens and Dr. W. R. Jenkins, Dr. H. J. Jensen, Mr. C. H. Keyser, Dr. J. W. Martin, Mr. C. W. McBeth, Dr. M. Oostenbrink, Mr. R. A. Rhode, Dr. J. W. Seinhorst and Dr. S. A. Sher contributed specimens of *Trichodorus* to the study, I am very grateful for their help and cooperation.

Morphology

The species of *Trichodorus* are small (0.5 to 1.5 mm. long), thick bodied, cylindrical nematodes tapering at the anterior end. Male and female tails are short and bluntly rounded. The cuticle is smooth, not marked by lines or punctations. In living specimens the cuticle is often observed to wrinkle as the nematode moves, giving the impression that the cuticle is rather loose. Fixed specimens frequently exhibit a swollen condition of the cuticle, this is apparently due to separation of the cuticle from the body during fixation. Because of this separation

of the cuticle measurements of body width are subject to error and variation. Since it is difficult to determine the thickness of the cuticle in fixed specimens cuticle thickness is not a reliable diagnostic character.

Body pores — Females may have a number of lateral pores that are valuable aids to identification. These pores vary in number and position in known species. In addition the known males and females of all but one species have paired subterminal caudal pores. Ventromedian pores are located near the vulva in one species, in another there are paired ventro-submedian pores near the vulva. The excretory pore is present in all known species.

Esophagus — Anteriorly a slender tube expanding gradually to form a pyriform or elongate conoid swelling that contains five esophageal gland nuclei.

Papillae — Sixteen cephalic papillae are present, an inner circle of six and an outer circle of ten. Males of all known species have a lateral cervical pore located near the level of the excretory pore. Paired adanal supplementary papillae are absent. However, all known males have a pair of postanal papillae and two pairs are present in some species. The ventromedian series of papillae varies from one to three in known species.

Spicula — Two spiculae varying in size among the species but usually elongate-slender and slightly curved.

Gubernaculum — Thin and relatively long, the distal end has a distinct keel in several species.

Caudal alae — Males of known species of *Trichodorus* represent two distinct groups, (1) males with caudal alae and (2) males without caudal alae. Associated with the presence of the caudal alae are rather obscure differences in the supplementary papillae and the shape of the spicules and the disposition of their musculature.

Female Reproductive System — The female reproductive system in eleven of the twelve species is typical of the didelphic systems of most dorylaims, the ovaries are reflexed and there is usually a spermatheca. However, the females of one species have a single anteriorly directed ovary that is not reflexed. In this species the posterior ovary is rudimentary and the vulva is located in the posterior half of the body . The cutinized pieces surrounding the vulva are frequently valuable diagnostic aids.

Onchiostyle — The onchiostyle of *Trichodorus* is generally referred to as being "slender and tripartite" and axial in position. This latter

character being used to designate the type of onchiostyle of the Dorylaimoidea other than the Nygolaimidae. Detailed study of the onchiostyle indicates that the *Trichodorus* do not have an axial onchiostyle. A study of cross-sections of the esophagus and the onchiostyle indicates (Fig. 2, A-1, 2, 3, 4 and 5) that the lumen of the esophagus lies ventral to the so-called axial spear and empties into the pharynx at the muscular collar previously thought to be a simple guiding ring. It is therefore probably proper to refer to the armature of the *Trichodorus* as a *dorsally* located *tooth*. This onchiostyle appears to be hollow for a distance anterior to the tripartite area, but it is not hollow throughout its length. In molting the anterior portion of the tooth from the tripartite area forward appears to be molted. The new onchiostyle is formed near the base of the old onchiostyle in the area surrounded by what appears to be a muscular sheath. These observations on the morphology indicate that although the onchiostyle may be used for puncturing cells, the actual feeding is accomplished by some mechanism other than through a hollow axial onchiostyle. Detailed observation of the feeding habits of these nematodes would undoubtedly contribute materially to an understanding of the morphology of the esophagus and the true function of the onchiostyle.

SYSTEMATICS

The genus *Trichodorus* is placed by THORNE (1939) in the subfamily Trichodorinae, family Diptherophoridae of the Dorylaimoidea. Although it appears that it would be justifiable to remove the Trichodorinae from this family, I prefer to leave the higher taxonomic categories in their present position until we have accumulated sufficient information to clearly define the relationships that exist among the Trichodorinae and the Diptherophorinae. Despite the great divergence of the onchiostyle in these subfamilies, close examination indicates that the Diptherophorinae do not have an axial onchiostyle, but have what may properly be regarded as a tooth since there is no aperture in the onchiostyle if one considers the onchiostyle to be the part that is molted (THORNE 1939).

The genus *Trichodorus* as it is constituted in this present study is composed of two species groups, in one group the males have caudal alae and in the other the caudal alae are absent. In some nematode groups the presence or absence of the caudal alae is considered to be of at least generic significance. I have, therefore, seriously considered

establishing two subgenera within the genus. However, while this procedure does indicate relationships within the genus I do not feel that it serves any other useful purpose. It would not, for example, facilitate the identification of species since I have been unable to discover any morphological differences that could be used to distinguish females of the two groups. In addition, males are not known for one species and they are extremely rare for several others. Until such time as the genus *Trichodorus* contains large numbers of described species and we have more knowledge of the food habits and biology of the species, I prefer not to establish subgenera.

Genus *Trichodorus* Cobb

Trichodorus Cobb, Jour. Wash. Acad. Sci., 3, 1913: 441.

Diagnosis — Dorylaimoidea, pharynx armed with a protrusible dorsal tooth or onchiostyle which is tripartite posterior to the middle. The onchiostyle is hollow for only a short distance anterior to the tripartite section. Amphid apertures conspicuous, elliptical. Esophagus enlarging to form a pyriform elongate bulb containing five esophageal gland nuclei. Ovaries one or two, in forms with two ovaries they are reflexed. Males with or without caudal alae. Paired adanal papillae not present. Spicules straight to slightly curved. Gubernaculum slender.

Type species — *Trichodorus primitivus* (de Man 1880).

Trichodorus monohystera, new species

(Fig. 1, A-E)

Dimensions. — 10 ♀s L = 0.61-0.90 mm., a = 15-23, b = 4.2-4.5, c = subterminal, V = $^{41\text{-}26}$ 77-83 $^{3.0\text{-}4.5}$ Onchiostyle 45-52 μ.

9 ♂s L = 0.67-0.90 mm., a = 19-24, b = 4.0-5.0, c = 70-90, T = 60-68 per cent. Onchiostyle 43-48 μ. Spicules = 51-59 μ. Gubernaculum = 10-13 μ.

Male (*Holotype*) — L = 0.9 mm., a = 19, b = 5.0, c = 90. Onchiostyle = 47 μ. Spicules = 58 μ. Gubernaculum = 12 μ. Excretory pore opening near the middle of the esophagus. A single ventromedian papilla just anterior to the excretory pore. A lateral pore opening slightly posterior to the level of the excretory pore. Three ventromedian supplementary papillae, the first just in front of the anal opening, the second at about the level of the proximal end of the spicules, the third about one body width anterior to the second. One pair of post-anal ventro-submedian papillae. Spicules slender, moderately curved. Gubernaculum linear.

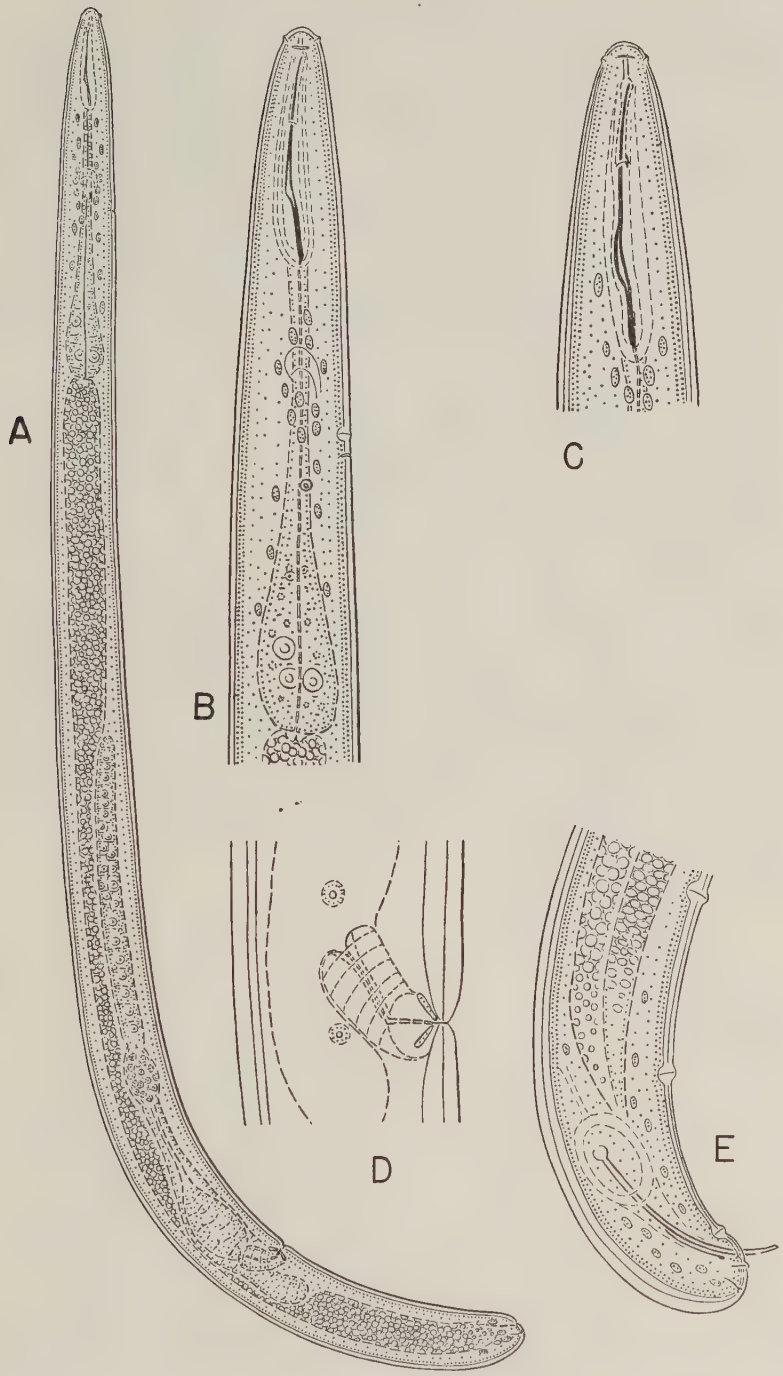

Fig. 1. *Trichodorus monohystera*: a, adult female, × 250; b, anterior end of male, × 500; c, female head, × 750; d, female vulva and lateral pores, × 750; e, male tail, × 500.

Female (*Allotype*) — L = 0.80 mm., a = 19, b, 4.8, c = subterminal, V = ^{38} 83 $^{3.2}$ Onchiostyle = 45 μ. Excretory pore located at the level of the middle of the esophagus. Single out-stretched anterior ovary. Spermatheca present. Posterior ovary vestigial, less than one body width in length. Two lateral pores, one slightly posterior to the level of the vulva and one less than a body width anterior to the vulva. Cutinized pieces surrounding the vagina elongated, slender. Inner portion of vagina directed anteriorly. Caudal pores subterminal.

Holotype — Male collected March 1955 by C. W. McBeth. Catalogue number 92, University of California Nematode Survey Collection, Berkeley.

Allotype — Female same data as holotype, Cat. No. 93, University of Californa Nematode Survey Collection.

Paratypes — 19 females and 24 males same data as holotype.

Type host — Soil at the roots of forest trees.

Type locality — Rancho Grande Road, Aragua, Venezuela. Elevation 1800 feet.

Males of *Trichodorus monohystera* can be distinguished by the shape of the spicules, the position of the three supplementary papillae and the presence of a single ventromedian papilla anterior to the excretory pore. Females of this species are distinguished by the single, out-stretched anterior ovary, the position of the vulva and the presence of two lateral pores near the level of the vulva.

Two females were collected from forest soil in the vicinity of the type locality but at an elevation of 2500 feet.

Trichodorus elegans, new species
(Fig. 2, A-D)

Dimensions. — 8 ♀ s L = 0.86-1,23 mm., a = 14-16, b = 4.0-4.8, c = subterminal, V = $^{25-17}$ 56-65$^{16-21}$. Onchiostyle = 140-150 μ.

5 ♂ s. L = 0.95-1.17 mm., a = 14-16, b = 4.0-4.7, c = 40-47, T = 55-63 per cent. Onchiostyle = 140-155 μ. Spicules = 55-62 μ. Gubernaculum = 20-22 μ.

Male (*Holotype*) — L = 1.1 mm., a = 15, b = 4.4, c = 44, Testis = 63 per cent. Onchiostyle = 140 μ. Spicules = 62 μ. Gubernaculum = 22 μ. Excretory pore opening at level of anterior end of the expanded portion of the esophagus. A single ventromedian papilla anterior to the excretory pore. A lateral hypodermal pore opening at the level of the ventromedian esophagal papilla. Three ventral sup-

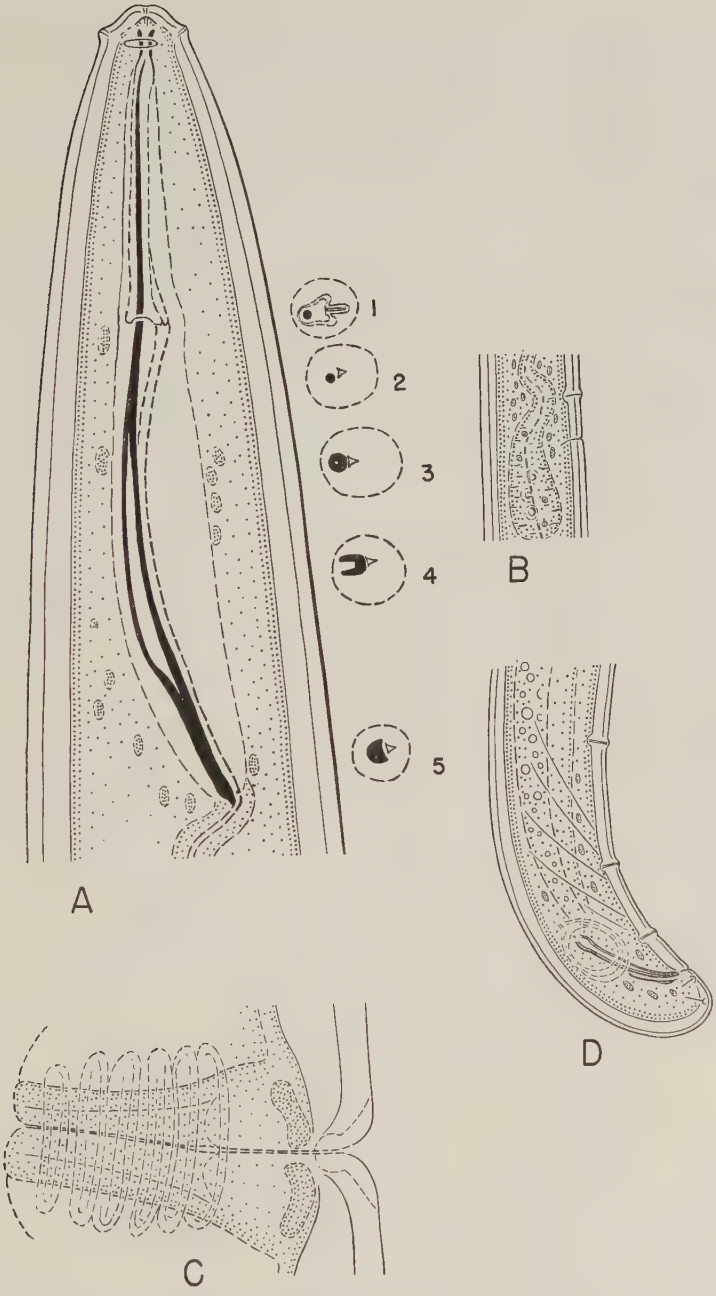

Fig. 2. *Trichodorus elegans:* a, female head, × 750; 1, 2, 3, 4, 5, cross sections through spear and esophagus, × 1500; b, posterior esophagus of male, × 250; c, female vulva, × 1500; d, male tail, × 750.

plementary papillae, the first located slightly posterior to the middle of the spicules, the second three fourths of a body width anterior to the first and the third about one body width anterior to the second. Paired postanal ventro-submedian papillae present. Caudal pores almost terminal in position. Spicules only slightly curved. Gubernaculum with a keel-like enlargement near distal end.

Female (*Allotype*) — L = 1.15 mm., a = 16, b = 4.6, c = subterminal, V = $^{21}60^{21}$. Onchiostyle = 140 μ. Excretory pore opening slightly anterior to middle of enlarged portion of esophagus. A single lateral hypodermal pore opening slightly posterior to the vulva. Spermatheca present. Anus subterminal. Caudal pores subterminal. Cutinized pieces surrounding vagina conspicuous, elongate-rounded in shape.

Holotype — Male collected July 1954 by Wm. K. Ferrell. Catalogue number 90. University of California Nematode Survey Collection, Berkeley.

Allotype — Female same data as holotype. Cat. No. 91. University of California Nematode Survey Collection, Berkeley.

Paratypes — 8 females and 2 males same data as holotype. Four females and 2 males collected by Wm. K. Ferrell at Nickle Plate Butte, Kaniksu National Forest, Idaho.

Type host — Soil at roots of pine.

Type locality — Moscow, Idaho.

Trichodorus elegans males can be distinguished from other species by the long onchiostyle, the position of the supplements and the single ventro-median esophageal papilla. Females are characterized by the long onchiostyle and the presence of a single lateral hypodermal pore slightly posterior to the vulva. This species appears most closely related to *T. obtusus* but is easily distinguished by the length of the onchiostyle and the presence of the ventromedian esophageal papilla anterior to the excretory pore in the male.

Trichodorus porosus, new species
(Fig. 3 A-F)

Dimensions. — 16 ♀ s L = 0.46-0.77 mm., a = 15-25, b = 4.1-5.5, c = subterminal, V = $^{28\text{-}19}53\text{-}58^{12\text{-}25}$. Onchiostyle = 43-50 μ. 11 ♂ s L = 0.53-0.77 mm., a = 15-25, b = 4.0-6.0, c = 60-90, T = 55-74 per cent. Onchiostyle = 43-48 μ. Spicules 36-39 μ. Gubernaculum = 12-13 μ.

Male (*Holotype*) — L = 0.62 mm., a = 19, b = 4.6, c = 83, Testis = 64 per cent. Onchiostyle = 48 μ. Spicules = 38 μ. Guber-

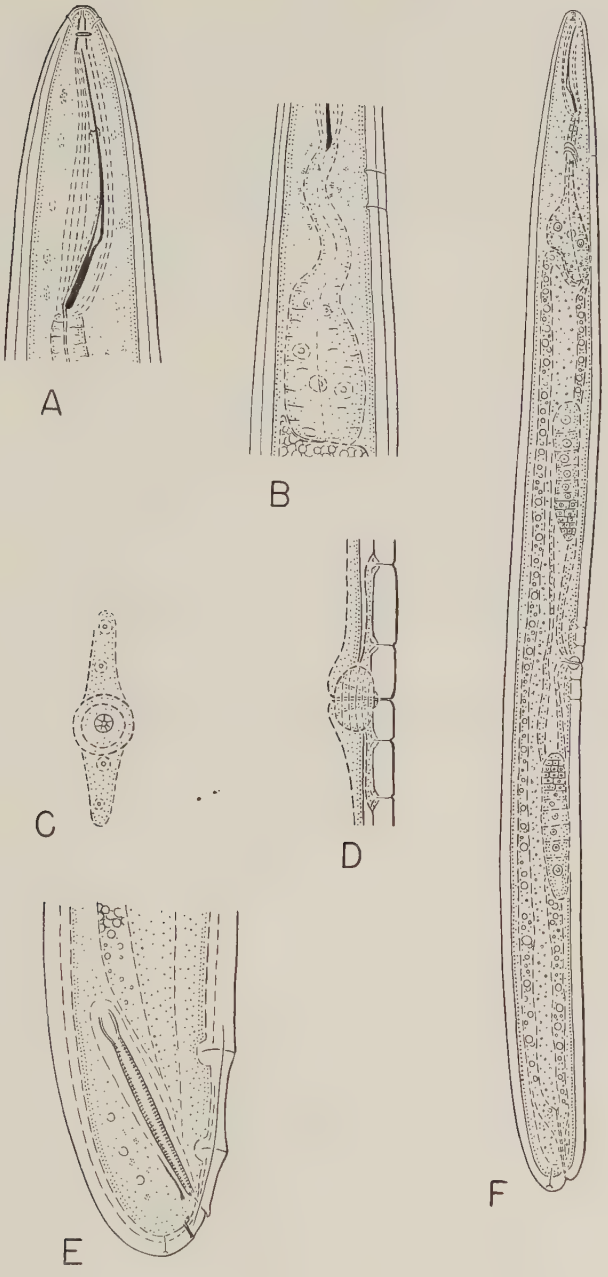

Fig. 3. *Trichodorus porosus*: a, female head, × 750; b, esophageal region of male, × 500; c, ventral view of vulva and ventro-median pores, × 1500; d, lateral view of vulva and ventro-median pores, × 1500; e, male tail, × 750; f, adult female, × 250.

naculum = 12 μ. Excretory pore opening at level of middle of esophagus. A single ventromedian papilla just anterior to the excretory pore. A lateral hypodermal pore opening at the level of the ventromedian esophageal papilla. Intestine slightly overlapping the posterior end of the esophagus. Two ventral supplementary papillae, both located posterior to the proximal end of the spicules. Paired postanal ventrosubmedian papillae present. Caudal pores almost terminal in position. Spicules straight, and having the appearance of being striated. Gubernaculum linear with the distal end slightly enlarged. Inconspicuous caudal alae present.

Female (*Allotype*) — L = 0.68 mm., a = 18, b = 5.0, c = subterminal. Onchiostyle = 50 μ. Excretory pore opening at level of middle of esophagus. Intestine overlapping the posterior end of the esophagus. A pair of ventro-median pores anterior and posterior to the vulva. Subterminal caudal pores present. Cutinized pieces surrounding the vagina, small and inconspicuous.

Holotype — Male collected December 14, 1954, by S. A. Sher. Catalogue Number 79, University of California Nematode Survey Collection, Berkeley.

Allotype — Female same data as holotype. Cat. No. 80. University of California Nematode Survey Collection, Berkeley.

Paratypes — Six females and fourteen males same data as holotype.

Type host — Soil at the roots of *Musa* sp.

Type locality — Corona, California.

Females of *Trichodorus porosus* can be distinguished by the position of the excretory pore and the presence of the pair of ventromedian pores posterior and anterior to the vulva. Males are characterized by the straight, striated spicules, two ventral supplementary papillae and the inconspicuous bursa. *T. porosus* differs from *T. pachydermus* and *T. christiei* in females having two pairs of ventromedian pores near the vulva and the males having two ventral supplementary papillae.

Males of *T. porosus* are rare and were present only in the collection from the type locality. The species has also been collected from soil at the roots of boysenberry and grape at Fresno, soil at the roots of *Musa cavendishii* at Oakland, soil at San Francisco, soil at the roots of Camellia at Sierra Madre and soil at boysenberry and walnut, Waterford, California. Soil at the roots of *Azalea* and *Ilex rotundifolia*, Spring Hill, Alabama, and soil at the roots of sugarcane and cabbage, Hawaii.

Trichodorus atlanticus, new species

(Fig. 4, A-E)

Dimensions. — 4 ♀ s L = 0.76-1.13 mm., a = 10-15, b = 5.5-6.3. c = subterminal. V = $^{27\text{-}21}$ 50-55$^{18\text{-}23}$. Onchiostyle = 64-70 μ.

3 ♂ s L = 0.93-1.2 mm., a = 12-19, b = 5.3-7.1, c = 41-57, T = 54-68 per cent. Onchiostlyle = 68-82 μ. Spicules = 79-87 μ. Gubernaculum = 18-20 μ.

Male (Holotype) — L = 1.2 mm., a = 19, b = 7.1, c = 41, Testis = 58 per cent. Onchiostyle = 82 μ. Spicules = 87 μ. Gubernaculum = 18 μ. Excretory pore opening at the anterior margin of the esophageal bulb. A single ventromedian papilla just anterior to the excretory pore. A lateral pore located at the level of the excretory pore. Ventromedian supplementary papillae three, two located within the bursal area and the third about four body widths from the end of the tail. Two pairs of submedian post-anal papillae. The caudal pores almost terminal. Spicules, slender and nearly straight. Gubernaculum slender and slightly enlarged at the distal end. A bilobed flap extending over the cloacal opening.

Female (Allotype) — L = 1.1 mm., a = 15.5, b = 5.8, c = subterminal V = ^{27}50^{19}. Onchiostyle = 82 μ. Excretory pore located near the level of the anterior end of the esophageal bulb. A pair of ventro-submedian pores located immediately posterior to the vulva. The opening of the vulva a longitudinal slit. Spermatheca present. Caudal pores nearly terminal.

Holotype — Male collected December 6, 1955 by D. J. Raski. Catalogue Number 97, University of California Nematode Survey Collection, Berkeley.

Allotype — Female same data as holotype. Catalogue number 98, University of California Nematode Survey Collection, Berkeley.

Paratypes — 3 females and 2 males same data as holotype.

Type host — Soil at the roots of sand pine.

Type locality — Winter Haven, Florida.

Males of *Trichodorus atlanticus* can be distinguished by the single ventromedian esophageal papilla, the position of the supplements and the presence of the bilobed flap covering the cloacal opening. Females can be distinguished by the presence of the ventro-submedian pores immediately posterior to the vulva.

This species has an exceedingly thick cuticle which loosens from the body when the nematode is fixed. Measurements of body diameter are

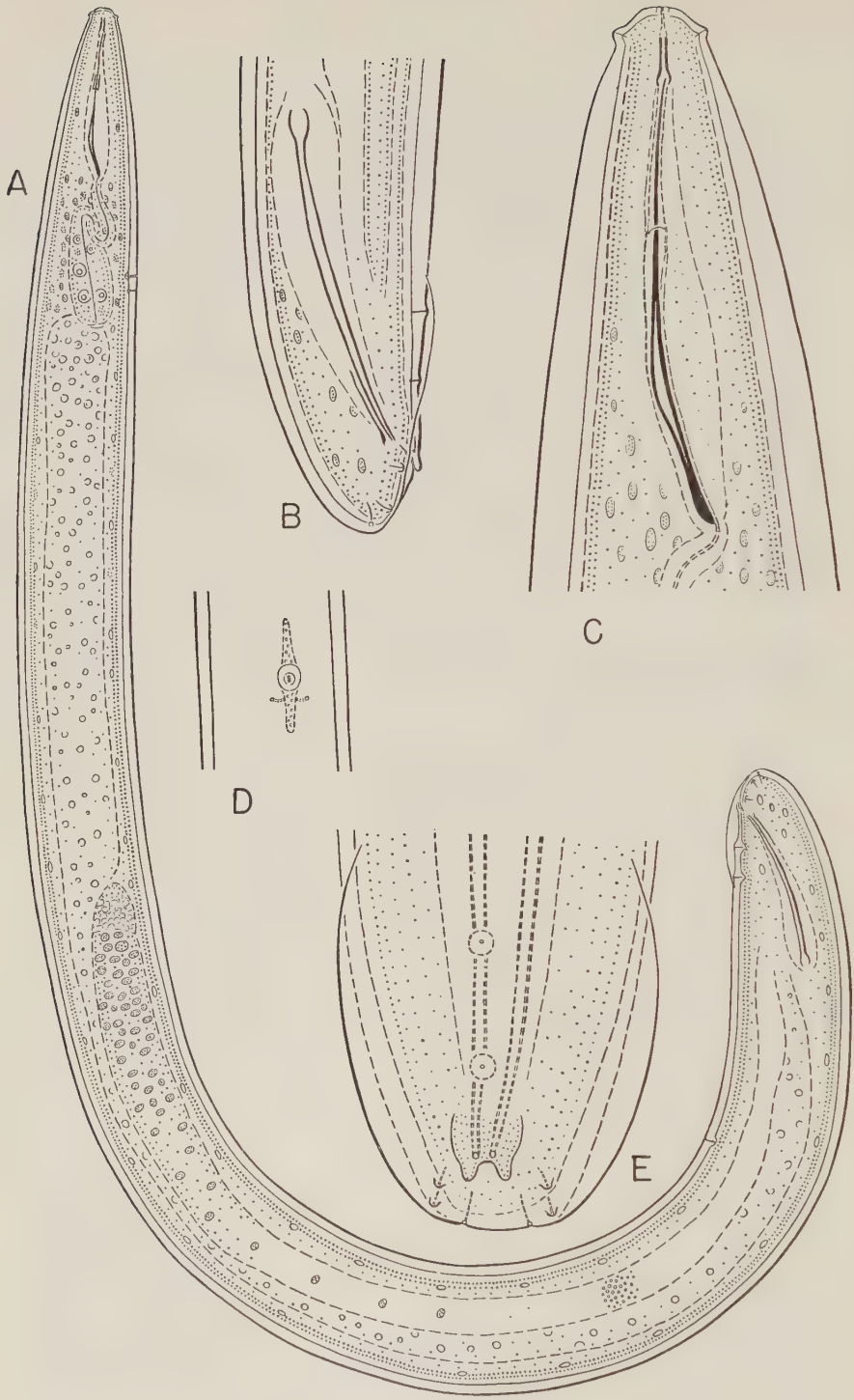

Fig. 4. *Trichodorus atlanticus*: a, adult male, × 250; b, male tail, × 500; c, female head, × 750; d, ventral view of vulva showing ventro-submedian pores, × 250; e, ventral view of male tail, × 750.

subject to error unless a correction is made for the distance the cuticle separates from the body.

Trichodorus christiei, new species
(Fig. 5 A-D)

Dimensions. — 20 ♀ s L = 0.46-0.71 mm., a = 15-20, b = 4.6-6.0, c = 110-140, V = $^{24\text{-}16}50\text{-}56^{13\text{-}26}$. Onchiostyle = 33-47 μ.

7 ♂ s L = 0.60-0.67 mm., a = 16-20, b = 5.0-6.5, c = 30-37, T = 50-67 per cent. Onchiostyle = 35-39 μ. Spicules = 60-65 μ. Gubernaculum = 18-20 μ.

Male (Holotype) — L = 0.62 mm., a = 17, b = 5.0, c = 35, testis, = 58 per cent. Onchiostyle = 37 μ. Spicules = 63 μ. Gubernaculum = 19 μ. Excretory pore opening near base of esophagus. Esophagus slightly overlapping intestine on ventral side. Lateral hypodermal pore and ventral esophageal papillae absent. A single ventral supplementary papilla located slightly anterior to anal opening. Paired caudal papillae opening just posterior to the caudal alae. Caudal alae well developed, not enclosing the terminus of the tail. Spicules elongate, slightly curved at the distal end. Spicules appearing to be striated. Gubernaculum linear. Caudal pores not observed.

Female (Allotype) — L = 0.55 mm., a = 15, b = 4.8, c = 125, V = $^{20}54^{15}$, Onchiostyle = 35 μ. Excretory pore opening near base of esophagus. Esophagus overlapping intestine. Spermatheca not observed. Cutinized pieces surrounding vagina small, inconspicuous. Lateral hypodermal pores and caudal pores absent.

Holotype — Male collected August 1954 by V. G. Perry. Catalogue number 81, University of California Nematode Survey Collection, Berkeley.

Allotype — Female collected October 17, 1952 by J. R. Christie. Catalogue number 82, University of California Nematode Survey Collection, Berkeley.

Paratypes — 5 males same data as holotype and 33 females same data as allotype.

Type host — Soil at the roots of celery.

Type locality — Sanford, Florida.

Females of *T. christiei* can be distinguished by the position of the excretory pore, esophagus overlapping the intestine, and by the absence of lateral hypodermal and caudal pores. The males are characterized by a well developed bursa, a single ventral supplementary pa-

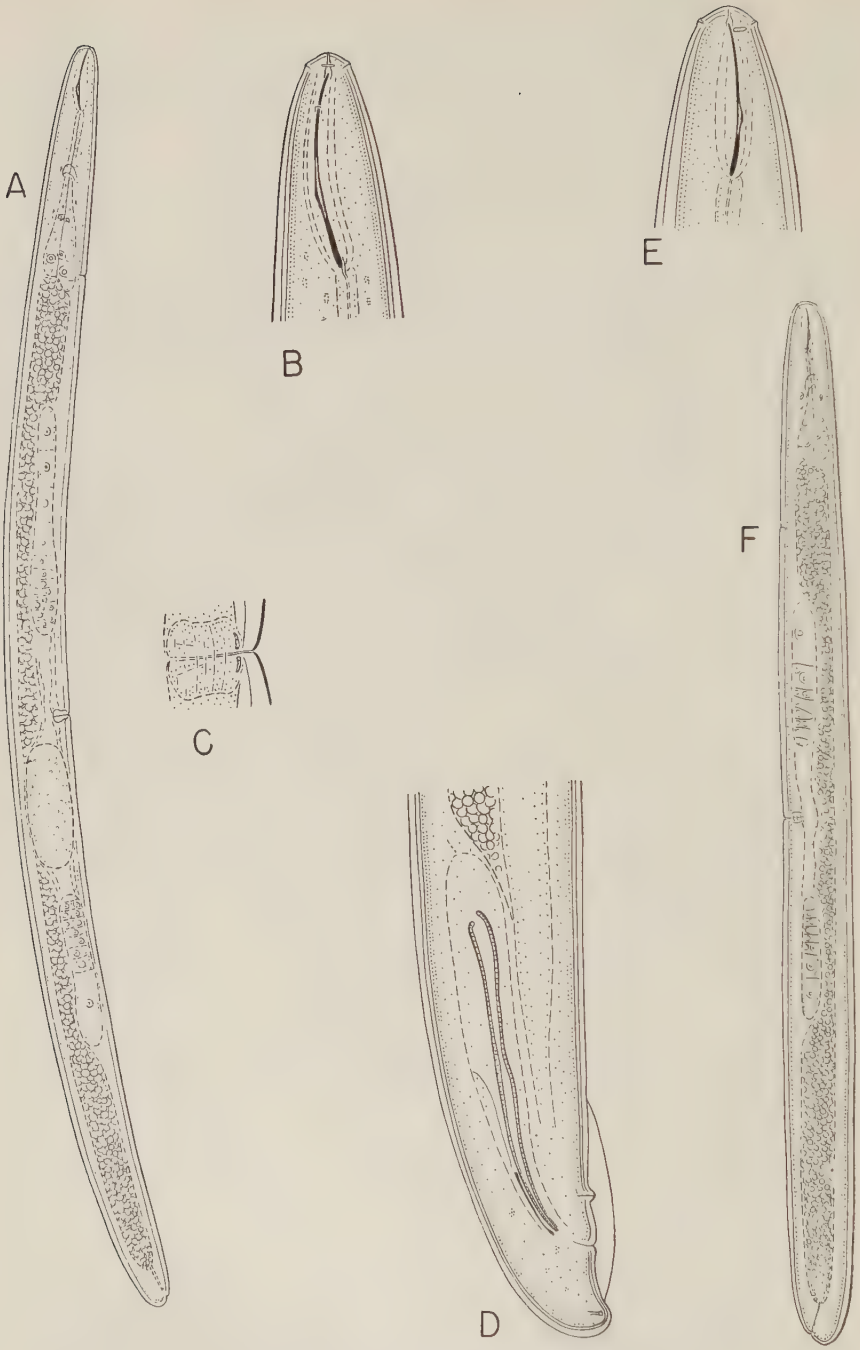

Fig. 5. *Trichodorus christiei*: a, adult female, × 500; b, female head, × 750; c, vulva, × 1500; d, male tail, × 750.
Trichodorus nanus: e, female head, × 750; f, adult female, × 250.

pilla and the long striated spicules. This species is separated from *T. pachydermus* by its smaller size, the absence of lateral hypodermal pores in the male and female and by the single supplementary papilla in the male. Males are very rare, a total of 8 having been found amongst several thousand females.

The species is named in honor of Dr. J. R. Christie, who, with Mr. V. G. Perry was the first to establish the pathogenicity of the species as an ectoparasite on the roots of plants. These authors suggested the common name, "stubby-root nematode", for this particular species because of the nature of the root symptoms caused by its feeding. It seems preferable to use this common name only for *T. christiei*, since no other species in the genus is known to produce the type of symptoms that are associated with the feeding of this species. The author is indebted to Mr. V. G. Perry for a collection containing six males of this species.

Trichodorus christiei appears to be the most widely distributed species of the genus in the United States. It has been collected in association with cultivated plants in several areas in the United States. Specimens were examined from soil taken at the roots of the following hosts at the indicated localities: Azalea and cotton, Alabama; alfalfa, boysenberry, cotton, grape, peach, persimmon, Strelitria and walnut, California; celery, tomato and St. Augustine grass, Florida; soil, Hawaii; artichoke and cotton, Louisiana; onion, Michigan; wheat, North Carolina; soil, Oregon; and blueberries, Salisbury, Maryland.

Trichodorus pachydermus Seinhorst
(Fig. 6, A-F)

Trichodorus pachydermus Seinhorst, Jour. Helm. 28, 1954: 111.

Dimensions — 14 ♀ s L = 0.66-0.99 mm., a = 16-28, b = 4.5-7.4, c = subterminal, V = $^{27\text{-}16}51\text{-}61^{13\text{-}29}$. Onchiostyle = 44-54 μ.

7 ♂ s L = 0.70-0.95 mm., a = 23-29, b = 4.4-6.4, c = 25-38, T = 54-63 per cent. Onchiostyle = 45-54 μ. Spicules = 44-54 μ. Gubernaculum = 12-16 μ.

Female — Excretory pore opening at about the level of the anterior end of the expanded portion of the esophagus. Esophagus not overlapping the intestine. Cutinized pieces surrounding vagina, small, elongate-rounded. Three lateral hypodermal pores posterior to the vulva. The first located about one body width posterior to the vulva, the second about one and three-fourths body widths posterior to the

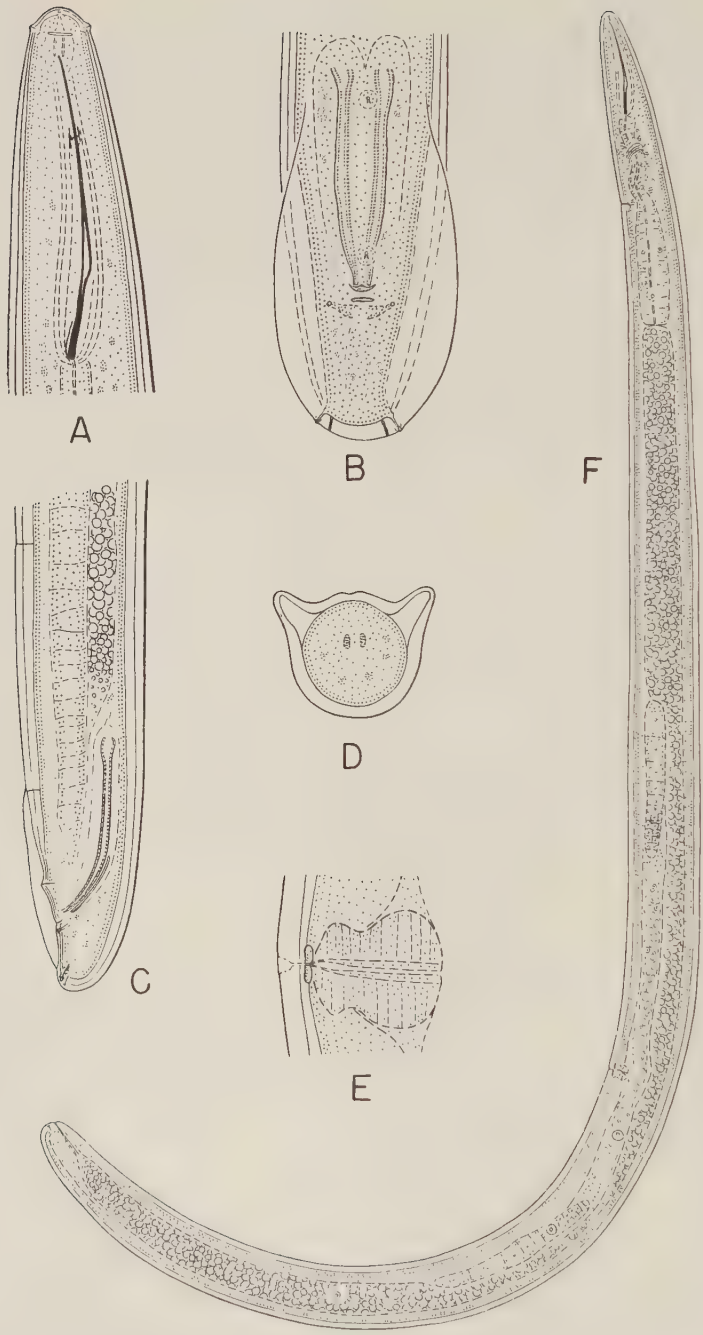

Fig. 6. *Trichodorus pachydermus*: a, female head, × 750; b, ventral view of male tail; × 750; c, lateral view of male tail × 500; d, cross-section through caudal alae, × 750; e, vulva, × 1500; f, adult female, × 250.

first and the third about one and three-fourths body widths posterior to the second. The caudal pores are subterminal in position.

Male — Excretory pore opening at the level of the beginning of the expanded portion of the esophagus. A single obscure ventral papilla located immediately anterior to the excretory pore. A lateral hypodermal pore opening slightly anterior to the level of the excretory pore. Three ventral supplementary papillae. One just anterior to the distal ends of the spicules, a second at the anterior beginning of the bursa and the third about two body widths anterior to the second. Two pairs of post-anal papillae present, the first pair near the anal opening, the second pair subterminal and near the caudal pores. The spicules are slightly curved, and have the appearance of being striated. The gubernaculum is slightly curved and slightly swollen at the distal end. Caudal alae present, beginning slightly posterior to the proximal end of the spicules and extending to the terminus of the tail.

Types — In the collection of the Institute for Phytopathological Research, Wageningen.

T. pachydermus is distinguished by the bursa, the number and position of the supplements, and the presence of a ventral esophageal papilla in the male. Females are characterized by the position of the excretory pore and the series of three lateral pores posterior to the vulva. These characters separate *T. pachydermus* from males and females of *T. porosus, T. christiei* and *T. atlanticus* which are the most closely related species.

In addition to the localities listed by Dr. J. W. Seinhorst, specimens were examined from soil at roots of oak, Beilen; soil, Gendringen; soil at roots of strawberry, Heemstede; soil at roots of *Crataegus* sp., Heiligerlee and Veendam; soil in meadow, Ruurlo; and soil at roots of beech tree and mulberry, Wageningen in the Netherlands and soil at turf, College Park, Maryland.

Trichodorus primitivus (de Man)
(Fig. 7, A-E)

Dorylaimus primitivus de Man, Tijdschr. Nederl. Dierk. Vereen. Deel V. 1880: 76.

Trichodorus primitivus, Micoletzky, Archiv. f. Naturg. 87. 1922: 539.

Trichodorus obtusus Cobb, Jour. Wash. Acad. Sci. 3. 1913: 441.

Dimensions. — 6 ♀ s L = 0.71-0.81 mm., a = 22-27, b = 4.9-5.5, c = subterminal, V = $^{22\text{-}19}55\text{-}57^{19\text{-}25}$. Onchiostyle = 45-52 μ.

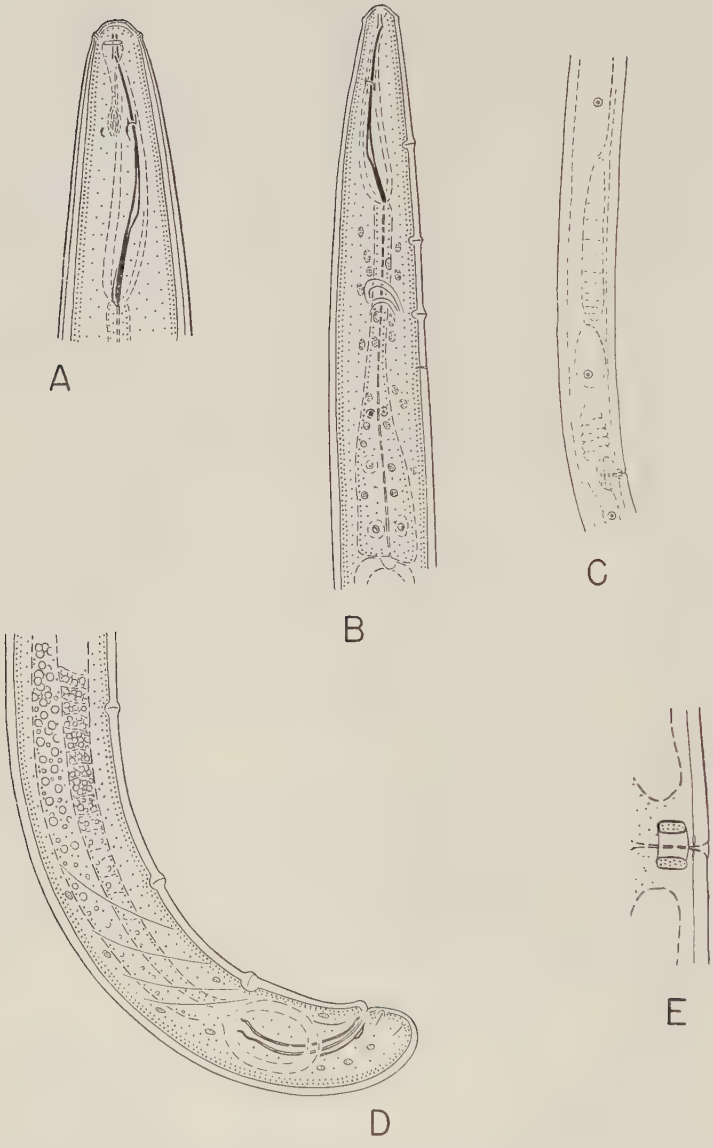

Fig. 7. *Trichodorus primitivus*: a, female head, × 750; b, anterior end of male, × 500; c, middle of female body showing location of lateral pores, × 250; d, male tail, × 500; e, vulva showing cutinized pieces, × 1500.

4 ♂ 5 L = 0.73-0.83 mm.; a = 22-26, b = 5.2-6.0, c = 55-61, T = 56-65 per cent. Onchiostyle 40-50 μ. Spicules = 33-40 μ. Gubernaculum = 15-18 μ.

Male (*Neotype*) — L = 0.83 mm., a = 25, b = 6.0 c = 55, Testis = 65 per cent. Onchiostlyle 47 μ. Spicules = 38 μ. Gubernaculum = 15 μ. Excretory pore opening just anterior to expanded portion of esophagus. Three ventromedian papillae located anterior to the excretory pore, one at the level of the posterior third of the onchiostyle, one slightly posterior to the base of the onchiostyle and one slightly posterior to the level of the nerve ring. A lateral hypodermal pore located at the level of the middle ventromedian papillae. Ventromedian supplementary papillae three, the first located near the proximal end of the spicules, the second one body width anterior to the first and the third one and three-fourth body widths anterior to the second. Paired postanal and caudal pores present. Spicules curved, gubernaculum with the distal end slightly enlarged.

Female — Excretory pore opening at the level of the nerve ring. Three lateral hypodermal pores, one slightly posterior to the vulva, one 3 body widths and one 7 body widths anterior to the vulva. Spermatheca present. Caudal pores subterminal, more ventrally located than in other species.

Neotype — Male collected December 1952, by J. W. Seinhorst. Catalogue Number 87, University of California Nematode Survey Collection, Berkeley.

Type host — Soil in Old Meadow.

Type locality — Ruurlo, Netherlands.

Males of *T. primitivus* can be distinguished from other species in the genus by the presence of three, or occasionally four, ventromedian esophageal papillae and the position of the ventral supplementary papillae. Females of this species can be recognized by the position of the excretory pore, the number and location of the lateral hypodermal pores, and the shape of the cutinized pieces surrounding the vulva. The female tail is longer than in most species and the caudal pores are more ventrally located.

Specimens of *T. primitivus* have been collected in Ruurlo, Wilhelminadorp and Veere, Netherlands and in a sugar beet field at Ballyculane, Ireland, soil at College Park, Maryland, and soil at the roots of boxwood, Philadelphia, Pennsylvania. *T. primitivus* was not found as frequently as *T. pachydermus* in collections from the Netherlands.

Trichodorus aequalis, new species
(Fig. 8, A-F)

Dimensions. — 8 ♀ s L = 0.62-0.95 mm., a = 17-22, b = 4.0-5.7, c = 170-186, V = $^{22\text{-}15}49\text{-}56^{15\text{-}26}$. Onchiostyle = 50-74 μ.

7 ♂ s L = 0.64-0.86 mm., a = 17-20, b = 4.5-6.4, c = 45-60, T = 56-70 per cent. Onchiostyle = 47-59 μ. Spicules = 35-50 μ. Gubernaculum = 14-23 μ.

Male (*Holotype*) — L = 0.84 mm., a = 17, b = 5.6, c = 60, Testis = 60 per cent. Onchiostyle = 56 μ. Spicules = 43 μ. Gubernaculum = 23 μ. Excretory pore opening near base of esophagus. Two ventromedian papillae located anterior to excretory pore. A lateral hypodermal pore located at about the level of the first ventromedian esophageal papilla. Ventromedian supplementary organs three, evenly spaced. The first located at the level of the proximal end of the spicules, the second about 1 body width and the third about two body widths anterior to the first supplement. Paired postanal ventrosubmedian papillae present. Caudal pores present, almost terminal in position.

Female (*Allotype*) — L = 0.75 mm., a = 18, b = 4.4, c = 186, V = $^{24}56^{26}$, Onchiostyle = 60 μ. Excretory pore located slightly posterior to middle of esophagus. Esophagus not overlapping intestine. Expanded posterior portion of esophagus about one-third the total length of esophagus including the spear. A lateral hypodermal pore located about four body widths anterior to vulva and one about one-half body width posterior to vulva. Spermatheca present. Caudal pores, almost terminal.

Holotype — Male collected January 27, 1952 by D. J. Raski and S. A. Sher. Catalogue Number 77, University of California Nematode Survey Collection, Berkeley.

Allotype — Female same data as holotype. Catalogue number 78, University of California Nematode Survey Collection, Berkeley.

Paratypes — One female same data as holotype.

Type host — Soil at the roots of toyon.

Type locality — Mt. Diablo, California.

Trichodorus aequalis can be distinguished by the two ventral esophageal papillae and the position of the supplements on the male tail. The females are distinguished from *Trichodorus proximus* by the position of the excretory pore and from *T. californicus* by the presence of a lateral hypodermal pore about four body widths anterior to the vulva.

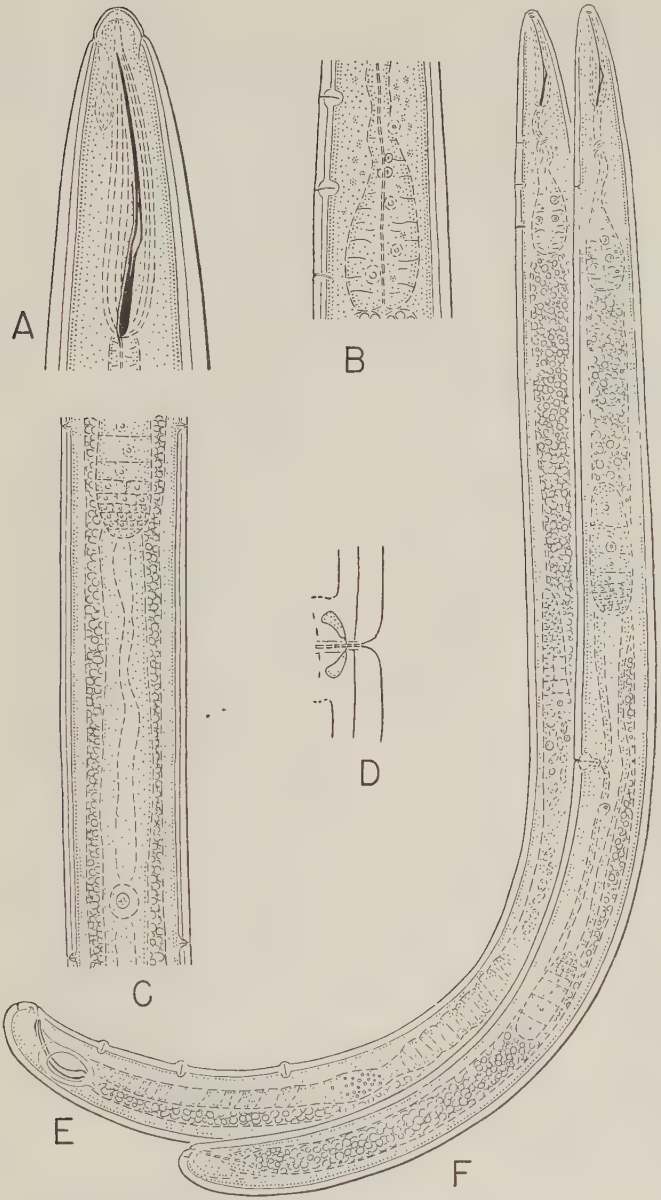

Fig. 8. *Trichodorus aequalis*: a, female head, × 750; b, posterior esophageal region of male, × 750; c, ventral view of vulva showing location of lateral pores, × 500; d, vulva, × 1500; e, male, × 250; f, female, × 250.

This species has been collected from soil at roots of manzanita, Baxter; *Arbutus* sp., Orinda; manzanita, Pyramid Ranger Station; *Eucalyptus*, Rancho Sante Fe in California. Soil at roots of pine and cactus, Idaho Springs, Colorado, and soil from Canby and Brooks, Oregon.

Trichodorus proximus, new species

(Fig. 9, A-E)

Dimensions. — 9 ♀ s L = 1.12-1.5 mm., a = 19-29, b = 7.8-9.3, c = < 250, V = $^{28\text{-}15}49\text{-}55^{15\text{-}33}$. Onchiostyle = 49-70 μ.

5 ♂ s L = 0.92-1.32 mm., a = 24-30, b = 6.6-7.5, c = 60-80, T = 56-63 per cent. Onchiostyle = 48-65 μ. Spicules = 48-65 μ. Gubernaculum = 18-25 μ.

Male (*Holotype*) — L = 1.29 mm., a = 29, b = 7.1, c = 80, Testis = 63 per cent. Onchiostyle 65 μ. Spicules = 65 μ. Gubernaculum = 25 μ. Excretory pore opening at level of middle of expanded portion of esophagus. A single ventromedian papilla less than a body width anterior to excretory pore. A lateral hypodermal pore opening at level of the ventromedian esophageal papilla. Three ventral supplementary papillae, the first located slightly anterior to the proximal end of the spicules, the second slightly more than a body width from the first and the third one and one-half body widths anterior to the second. Paired postanal ventro-submedian papillae present. Caudal pores present, almost terminal in position.

Female (*Allotype*) — L = 1.42 mm., a = 28, b = 9.0, c = 300, V = $^{21}55^{21}$, Onchiostyle = 70 μ. Excretory pore opens slightly anterior to base of esophagus. Esophagus not overlapping intestine. A lateral hypodermal pore located about 3 body widths anterior to the vulva and one about one half body width posterior to vulva. Spermatheca present. Anus almost terminal. Caudal pores, almost terminal in position.

Holotype — Male collected November 4, 1952 by J. R. Christie. Catalogue Number 83, University of California Nematode Survey Collection, Berkeley.

Allotype — Female same data as holotype. Catalogue Number 84, University of California Nematode Survey Collection, Berkeley.

Paratypes — One female and one male same data as holotype.

Type host — Soil at the roots of St. Augustine grass.

Type locality — Tampa, Florida.

Trichodorus proximus can be distinguished by the single ventral

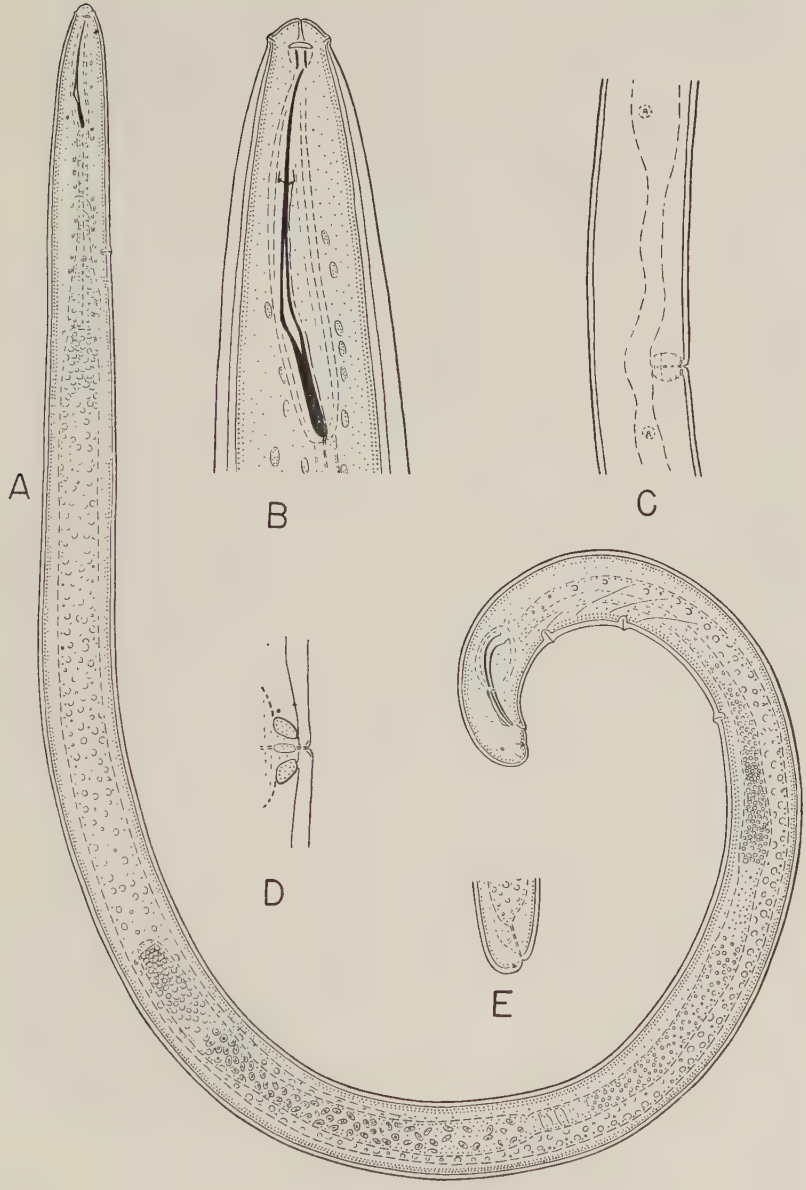

Fig. 9. *Trichodorus proximus*: a, adult male, × 250; b, female head, × 750; c, lateral view at middle of body showing vulva and location of lateral pores, × 250; d, vulva and cutinized pieces, × 1500; e, female tail, × 250.

esophageal papilla and the position of the supplements on the male tail. The female of this species can be separated from *T. aequalis* and *T. californicus* by its larger size, position of the excretory pore and the shape of the cutinized pieces surrounding the vulva.

The species has also been collected from soil around the roots of Bermuda grass at Gainesville, Florida.

Trichodorus californicus, new species
(Fig. 10, A-F)

Dimensions. — 13 ♀ s L = 0.79-1.25 mm., a = 15-20, b = 4.4-6.2, c = subterminal V = $^{27\text{-}19}57\text{-}61^{14\text{-}26}$, Onchiostyle = 55-80 μ.

11 ♂ s L = 0.67-1.06 mm., a = 15-21, b = 4.2-6.6, c = 44-58, T = 60-75 per cent, Onchiostyle = 54-82 μ. Spicules = 43-70 μ. Gubernaculum = 20-32 μ.

Male (*Holotype*) — L = 1.06 mm., a = 21, b = 6.6, c = 51, Testis = 71 per cent. Onchiostyle = 82 μ. Spicules = 70 μ. Gubernaculum = 32 μ. Excretory pore opening at level of the beginning of the expanded portion of the esophagus. A single ventromedian papilla located less than one body width anterior to the excretory pore. A lateral hypodermal pore opening at the level of the ventromedian esophageal papilla. Three ventral supplementary papillae on tail. The first located just anterior to the cloacal opening, the second at the level of the proximal end of the spicules and the third less than one body width anterior to the second. Paired post-anal ventro-submedian papillae present. Caudal pores almost terminal in position.

Female (*Allotype*) — L = 1.01 mm., a = 17, b = 5.0, c = subterminal V = $^{25}58^{26}$, Onchiostyle = 79 μ. Excretory pore opening at the level of the beginning of the expanded portion of the esophagus. Esophagus not overlapping the intestine. A single lateral hypodermal pore less than one body width posterior to the level of the vulva. Spermatheca present. Anal opening almost terminal. Caudal pores almost terminal in position.

Holotype — Male collected March 30, 1952 by D. J. Raski. Catalogue number 85, University of California Nematode Survey Collection, Berkeley.

Allotype — Female, same data as holotype. Cat. No. 86, U. C. Nematode Survey Collection, Berkeley.

Paratypes — Four females and one male, same data as holotype.

Type host — Soil at the roots of *Pinus attenuata.*

Type locality — Moraga Ridge, Contra Costa County, California.

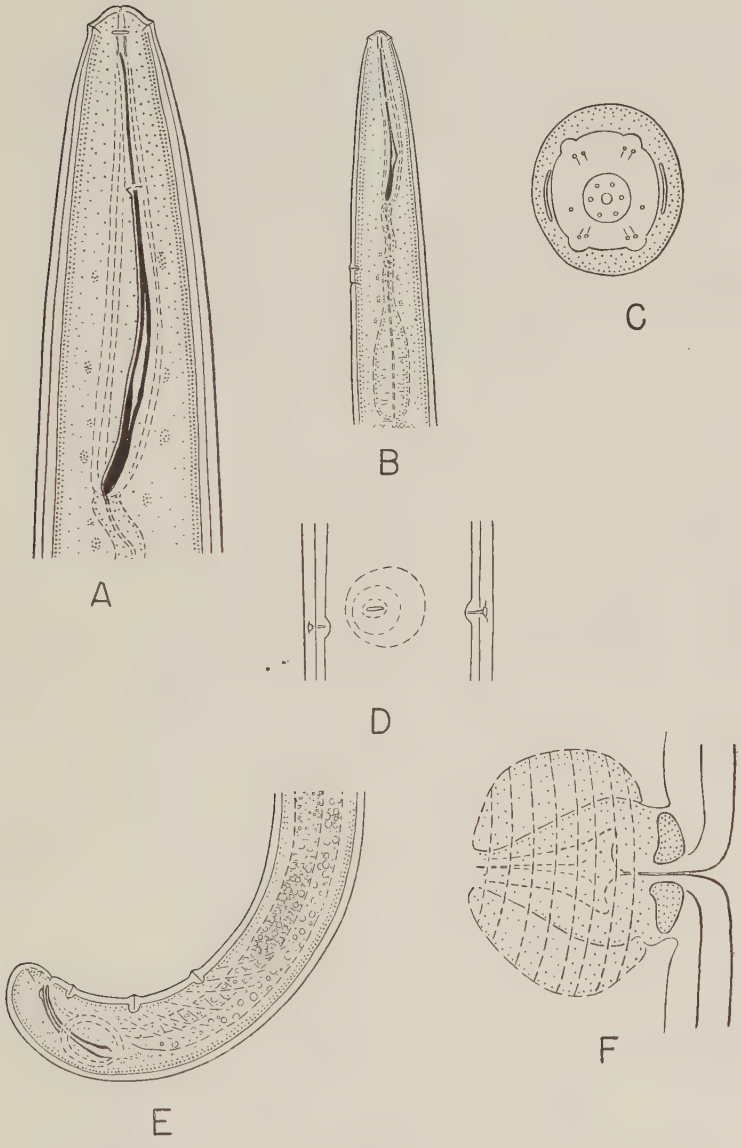

Fig. 10. *Trichodorus californicus*: a, female head, × 750; b, esophageal region of male, × 250; c, face view of female, × 1500; d, ventral view of vulva showing location of lateral pores, × 500; e, male tail, × 250; f, vulva, × 1500.

Trichodorus californicus can be distinguished by the single ventral esophageal papilla and the position of the ventral supplements on the male tail. Females of this species can be separated from females of *T. aequalis* by the shape of the cutinized pieces surrounding the vulva and by the absence of lateral hypodermal pores anterior to the vulva. *T. californicus* females differ from females of *T. proximus* in having a single lateral hypodermal pore, being smaller in size and in the shape of the cutinized pieces surrounding the vulva.

This species has been collected from soil at the roots of *Mesembryanthemum,* Dillon Beach; soil at the roots of oak, Bolinas Bay; soil at the roots of redwood, Garberville and Richardson's Grove; soil at the roots of Bay tree, Orinda, and Jenner; soil at the roots of willow, Loomis in California; and soil at the roots of Conifer, Echo Lake, Colorado.

Trichodorus nanus, new species

(Fig. 5, E-F, page 46)

Dimensions. — 6 ♀s L = 0.49-0.55 mm., a = 12.8-16, b = 5.1-6.1, c = 87, V = $^{24\text{-}14}49\text{-}54^{14\text{-}20}$. Onchiostyle 22-23 μ.

Female (*Holotype*) — L = 0.52 mm., a = 16, b = 5.4, c = 87, V = $^{17}53^{14}$, Onchiostyle 22 μ. Excretory pore opening one and one-half body widths posterior to base of esophagus. Esophagus not overlapping intestine. Spermatheca not present. Cutinized pieces surrounding vulva, rather small, inconspicuous. No lateral hypodermal pores or subterminal caudal pores. Males of this species not known.

Holotype — Female collected in 1952 by J. W. Seinhorst. Catalogue Number 88, University of California Nematode Survey Collection, Berkeley.

Paratypes — 11 females same data as holotype.

Type host — Soil at the roots of rye.

Type locality — Grave, Netherlands.

Females of *T. nanus* are easily distinguished from other species of the genus by their small size, the location of the excretory pore and the absence of subterminal caudal pores. This species resembles *T. christiei* in not having caudal pores, but is easily recognized by the position of the excretory pore and the short spear.

Specimens were collected from soil at the roots of rye, Grave; soil at the roots of strawberry, Heemstede; and soil, Wageningen in the Netherlands. This was a rare species in samples collected in the Netherlands, it was found in only three of 133 samples.

Trichodorus obscurus, new species
(Fig. 11, A-C)

Dimensions. — 9 ♀ s L = 0.72-1.17 mm., a = 16-24, b = 4.5-6.3, c = subterminal V = $^{27\text{-}21}54\text{-}58^{16\text{-}28}$. Onchiostyle 60-72 μ.

7 ♂ s L = 0.69-1.04 mm., a = 14-22, b = 4.2-5.7, c = 55-69. T = 61-70 per cent. Onchiostyle = 60-77 μ. Spicules = 50-62 μ. Gubernaculum = 20-22.5 μ.

Male (*Holotype*) — L = 1.05 mm., a = 21, b = 5,5, c = 60, Testis = 62 per cent. Onchiostyle = 60 μ. Spicules = 58 μ. Gubernaculum = 21 μ. Excretory pore opening near the anterior margin of the esophageal bulb. No ventromedian papilla anterior to excretory pore. A lateral hypodermal pore located just anterior to the excretory pore. Ventromedian supplementary papillae three, the first near the distal end of the spicules, the second near the proximal end of the spicules, the third less than one body width anterior to the second. Paired post-anal ventro-submedian papillae present. Caudal pores almost terminal in position.

Female (*Allotype*) — L = 1.17 mm., a = 21, b = 6.3, c = subterminal, V = $^{25}56^{25}$ Onchiostyle = 66 μ. Excretory pore opening at the base of the corpus of the esophagus. A single lateral pore located at the level of the vulva. Spermatheca present. Caudal pores almost terminal in position.

Holotype — Male collected April 8, 1931 by Gerald Thorne. Catalogue number 94, University of California Nematode Survey Collection, Berkeley.

Allotype — Female same data as holotype. Catalogue number 94, University of California Nematode Survey Collection, Berkeley.

Paratypes — Seven females and seven males same data as holotype.

Type host — Soil at the roots of grass sod.

Type locality — Arlington Farm, Virginia.

Trichodorus obscurus can be distinguished by the absence of ventral esophageal papillae and the position of the supplementary papillae on the male tail. The females of this species cannot be distinguished from those of *T. californicus*.

This species has been collected from soil at the roots of Manzanita 10 miles south of Monticello and Shasta Lake California, and soil at Brookings and Seal Rock, Oregon, and sod at Arlington, Virginia.

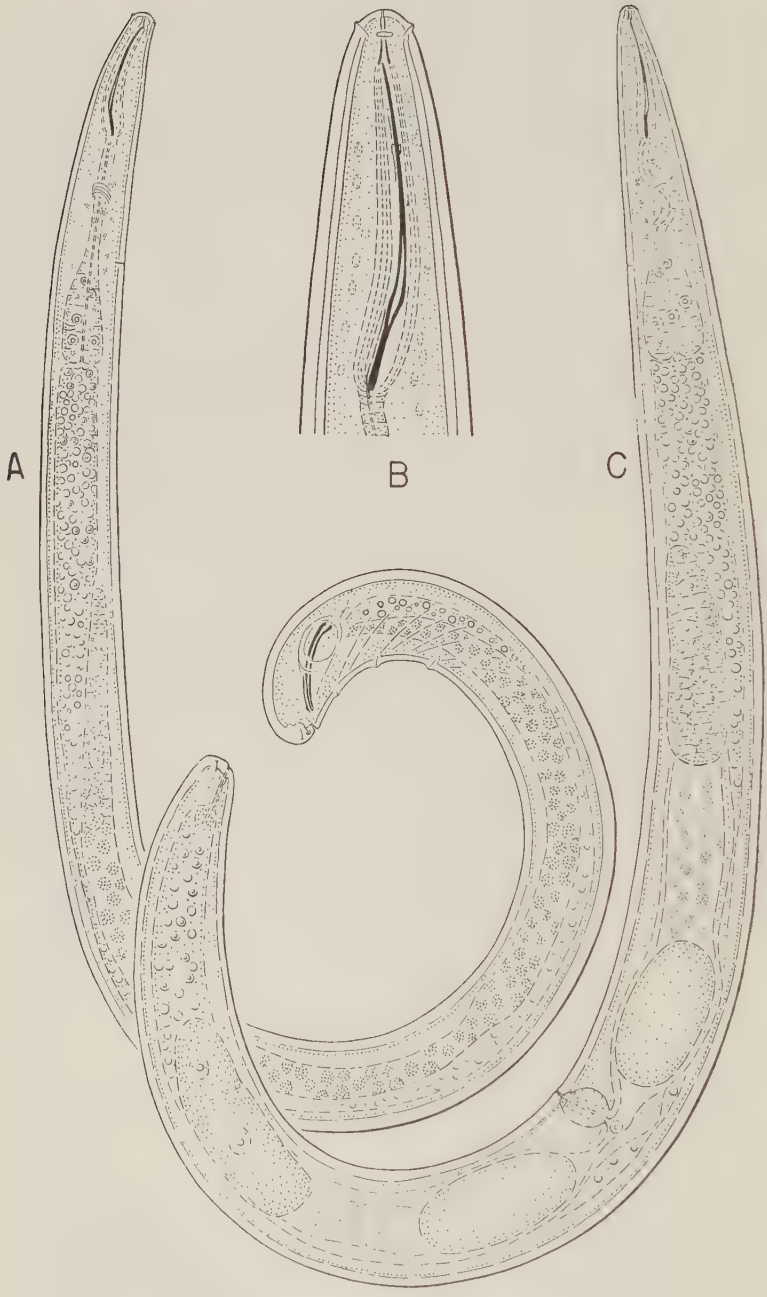

Fig. 11. *Trichodorus obscurus*: a, adult male, × 250; b, female head, × 750; c, adult female, × 250.

Key to the species of *Trichodorus* (males and females)

1. Females with one ovary (monodelphic) . . . . *monohystera* n. sp.
   Females with two ovaries (amphidelphic) 2
2. Spear more than 130 microns long . . . . . *elegans* n. sp.
   Spear less than 110 microns long 3
3. Excretory pore posterior to base of the esophagus, spear less than 30 microns long, males not known . . . . . *nanus* n. sp.
   Excretory pore anterior to end of esophagus, spear more than 30 microns long, males known 4
4. Females with ventro-median or ventro-submedian pores near vulva, males with 2 or 3 supplementary papillae 5
   Females without ventro-median or ventro-submedian pores near vulva, males with one or 3 supplementary papillae 6
5. Females with ventro-median pores, 2 anterior and 2 posterior to the vulva, male with caudal alae, 2 supplementary papillae . . . *porosus* n. sp.
   Females with 2 ventro-submedian pores posterior to the vulva, male with caudal alae, 3 supplementary papillae . . . . *atlanticus* n. sp.
6. Females without caudal pores or lateral hypodermal pores, males with a single supplementary papilla, caudal alae present . . *christiei* n. sp.
   Females with caudal pores and lateral hypodermal pores, males with or without caudal alae, more than one ventral supplement 7
7. Females with three lateral hypodermal pores posterior to vulva, males with caudal alae and three ventral supplements . . *pachydermus* Seinhorst
   Females with one lateral hypodermal pore posterior to vulva, males without caudal alae 8
8. Females with three lateral hypodermal pores, 2 anterior and one posterior to vulva, males with 3 or 4 ventro-median papillae anterior to excretory pore *primitivus* (de Man)
   Females with not more than two lateral hypodermal pores, males with 0-2 ventro-median papillae anterior to excretory pore 9
9. Females with one lateral hypodermal pore posterior and one anterior to vulva, 1st supplement in male about opposite proximal end of spicules 10
   Females with one lateral hypodermal pore at level of vulva, males with 1st supplement near distal end of spicules 11
10. Excretory pore in female slightly posterior to middle of esophagus, males with 2 ventro-median esophageal papillae anterior to the excretory pore . *aequalis* n. sp.
    Excretory pore in female near base of esophagus, male with one ventro-median papilla anterior to excretory pore . . . *proximus* n. sp.
11. Male with one ventro-median papilla anterior to excretory pore . . . *californicus* n. sp.
    Male without ventro-median esophageal papilla . . *obscurus* n. sp,

SUMMARY

Trichodorus primitivus (de Man 1880) is redescribed and ten new species of *Trichodorus* are described, including the species first reported to cause stubby-root disease in Florida, U.S.A. The species descriptions and illustrations present details of the morphology of both males and females and indicate the presence of two distinct species groups in the genus. The morphology of the onchiostyle is discussed and illustrated and evidence is presented to indicate that the onchiostyle

in *Trichodorus* is not a hollow axial spear but is dorsal to the lumen of the esophagus. A key to the species is presented as an aid to identification.

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NEMATODEN AN BAUMSCHULGEWÄCHSEN IN MITTELDEUTSCHLAND *)

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Über die Ursachen der sogenannten „Baumschulmüdigkeit" wird zur Zeit heftig diskutiert. Mehrere Erklärungsversuche liegen vor und die bisherigen Ergebnisse lassen zweifellos die vorläufige Schlussfolgerung zu, dass das gleiche Erscheinungsbild verschiedene Ursachen haben kann. Durch zahlreiche Untersuchungen ist nachgewiesen, dass als eine dieser Ursachen parasitische Nematoden anzusprechen sind.

Dass Nematoden als Wurzelschädlinge von Holzgewächsen eine Rolle spielen, ist seit langem bekannt, dass ihnen für eine „Baumschulmüdigkeit" eine Bedeutung zukommt, wurde von OOSTENBRINK (1955) eindeutig nachgewiesen. In dieser Veröffentlichung ist auch die Literatur weitestgehend berücksichtigt worden. Es sei darüber hinaus noch verwiesen auf: FISCHER (1955), LOWNSBERY (1956), MILOVTSOVA (1953), sowie WEISCHER (1956).

Wir haben seit dem Herbst 1955 eine grössere Anzahl mitteldeutscher Baumschulen, in denen sich Nachbauschwierigkeiten zeigen, daraufhin untersucht, inwieweit Nematoden als Ursache in Frage kommen. Dabei hat sich gezeigt, dass in einigen Fällen den Nematoden Bedeutung beizumessen ist, dass jedoch in anderen Baumschulen auch andere Ursachen wahrscheinlich sind.

Über einige Ergebnisse unserer noch laufenden Untersuchungen wird hier berichtet.

I. Methodik

Bodenproben, Wurzelstücke und einzelne Sämlinge wurden aus gesunden und aus auffallend kranken Baumschulquartieren entnommen. Da ektoparasitische und freilebende Nematoden sehr stark auf

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Milieuveränderungen reagieren und entweder abwandern oder beim Herausnehmen aus der Erde von den Wurzeln abfallen können, wurden die Proben in gut verschliessbaren Glaszylindern transportiert, und um ein möglichst genaues Spiegelbild der natürlichen Verhältnisse in den Baumschulen zu bekommen, möglichst unverzüglich verarbeitet. Die Bodenproben wurden nach der Baermann-Methode ausgewaschen, die sich im Schlauch ansammelnden Älchen wurden nach 8-12 Stunden ausgezählt. Die mitgeteilten Zahlen beziehen sich stets auf 10 ccm Boden. Wir haben das Raummass gewählt, da das spezifische Gewicht der einzelnen Bodenarten grundverschieden sein kann; 1 g eines leichten Bodens sind z.B. etwa 3-5 ccm, während 1 g eines schweren Bodens nur 1-2 ccm füllen.

Für die Untersuchung der Wurzeln, denen wir grosse Bedeutung beimessen, wurden von den Faserwurzeln, den Seitenwurzeln und der Hauptwurzel bzw. dem unter der Erde liegenden Wurzelhals 10 cm mit dem noch daran haftenden Boden in einer halb mit Wasser gefüllten Petrischale vorsichtig zerzupft. Nach 8-12 Stunden schütteten wir das Wasser aus den Schalen in mit kleinen Gazebeuteln ausgekleidete Baermann-Trichter. Nach einigen Stunden konnten die Älchen ausgezählt werden, ohne dass Schmutzteilchen die Arbeit behinderten.

Unter den obligaten Parasiten waren die Arten der Gattungen *Pratylenchus, Rotylenchus* und *Ditylenchus* besonders stark vertreten. Sie wurden in den Tabellen mit den anderen Vertretern der gleichen Familie als Tylenchidae zusammengefasst. Daneben werden die ebenfalls parasitischen Dorylaimidae aufgeführt. Die saprozoitischen Rhabditidae und alle Arten aus anderen Familien wurden unter „Sonstige" eingereiht.

II. Ergebnisse

Nematoden an Obstgehölzen.

Besondere Aufmerksamkeit wurde in den letzten Jahren dem Befall von Wurzeln der Obstgehölze gewidmet, wobei sich der Begriff „Obst" auf Kern-, Stein-, Beeren- und Schalenobst, auf Wein, auf Südfrüchte, auf die als Unterlagen in Frage kommenden Wildformen und auf die mit den Kulturarten nahe verwandten Wildarten bezieht. Wir haben mehrere Obstbaumschulen mit Müdigkeitserscheinungen untersucht. Dabei erhielten wir unterschiedliche Ergebnisse in bezug auf den Nematodenbesatz des Bodens und der Wurzeln, die es wahrscheinlich machen, dass nicht in jedem Fall Nematoden für die Nach-

bauausfälle verantwortlich gemacht werden können. In einigen Baumschulen, vor allem in solchen auf stark humuszehrenden Böden wurden jedoch aus dem Boden und aus den Wurzeln grössere Zahlen an obligat-parasitischen Nematoden ausgewaschen, die eine Einwirkung dieser Schädlinge nicht zweifelhaft erscheinen lassen. Die für einen Nematodenbesatz typischen bräunlichen Nekrosen der Wurzelepidermis und die geringe Faserwurzelbildung vervollständigten das Bild.

Nematoden an anderen Laubgehölzen.

Über Nematodenfunde an und in Wurzeln von Jungpflanzen anderer Laubgehölze liegen ebenfalls eine Reihe von Mitteilungen vor. Wir konnten eine Baumschule in Bad-Liebenwerda mit stark „baumschulmüden" Quartieren von Laubgehölzen untersuchen. Dabei stellten wir auf allen geschädigten Flächen Nematoden, hauptsächlich *Pratylenchus-*, *Rotylenchus-* und *Dorylaimus*-Arten fest (Tab. I). Die Zah-

TABELLE I

Laubgehölze

Bodenverhältnisse: Mineral. Nassboden, lehm. Sand, Raseneisenstein.
Jährl. Niederschlagssumme: 600-650 mm, hoher Grundwasserstand.
Probeentnahme: Ende September.

	Stand	Nematoden in 10 ccm Boden und (in Klammern) auf 10 cm Wurzellänge *Tylenchidae*	*Dorylaimidae*	Sonstige	insgesamt
1. Robinia pseudacacia	schlecht	13 (17)	2 (2)	5 (13)	20 (32)
	gut	0 (1)	0 (0)	2 (4)	2 (5)
2. Caragana arborescens	schlecht	16 (19)	4 (2)	2 (13)	22 (34)
	gut	4 (4)	2 (0)	1 (2)	7 (6)
3. Prunus padus	schlecht	13 (15)	4 (2)	8 (12)	25 (29)
	gut	0 (1)	0 (0)	0 (3)	0 (4)
4. Laburnum anagyroides	schlecht	46 (16)	9 (2)	6 (11)	60 (29)
	gut	3 (0)	0 (0)	5 (2)	8 (2)
5. Sambucus rubra	schlecht	14 (25)	4 (3)	12 (17)	30 (45)
	gut	0 (2)	0 (1)	0 (25)	0 (28)
6. Rosa pollmariana	schlecht	12 (30)	6 (2)	11 (9)	29 (41)
	gut	0 (0)	0 (0)	2 (2)	2 (2)
7. Syringa vulgaris	schlecht	15 (14)	3 (2)	9 (11)	27 (27)
	gut	0 (0)	0 (0)	0 (1)	0 (1)

len aus den Bodenproben sind unterschiedlich, die hohe Zahl für Laburnum fällt zweifellos auf. Es muss jedoch dazu erwähnt werden, dass die Untersuchung der Bodenproben aus dem Laburnum-Quartier sofort erfolgte, während die übrigen Proben erst nach 8 Tagen verarbeitet werden konnten.

Die Zahlen machen eine Korrelation zwischen „Baumschulmüdigkeit" und Nematodenbesatz sehr wahrscheinlich, vor allem spricht dafür die Tatsache, dass *Pratylenchus*-Arten in den Wurzeln der Pflanzen aus schlechten Quartieren vorherrschten. *Rotylenchus*-Arten wurden stets in den Bodenproben in grösserer Zahl gefunden, von den Wurzeln aber nur vereinzelt isoliert, was sich daraus erklärt, dass sich diese Arten bei Erschütterungen von den Wurzeln abfallen lassen.

Die hohe Gesamtzahl an Nematoden in den Wurzeln bei Holunder auf Flächen mit gutem Stand ist auf einen erheblichen Besatz mit den ungefährlichen Rhabditiden zurückzuführen. Die wirklich schädlichen Tylenchiden und Dorylaimiden wurden in den „gesunden" Holunderquartieren nur im gleichen Verhältnis festgestellt wie bei den anderen Holzarten. Die Ursachen für den hohen Rhabditiden-Besatz, der bisher in ähnlicher Weise bei keiner anderen Pflanzenart beobachtet wurde, sind noch ungeklärt.

Das Schadbild glich dem für die Obstgehölze beschriebenen. Die Wurzeln hatten infolge des Fehlens von Faserwurzeln ein struppiges Aussehen, die wenigen noch vorhandenen Faserwurzeln waren dicht mit nekrotischen Flecken bedeckt.

Nematoden an Nadelhölzern.

Über Nematodenschäden an Nadelhölzern wurde bisher aus den USA von Steiner (1949), Henry (1953) und Milovtsova (1953) und aus Holland von Oostenbrink (1955) berichtet. In Deutschland konnten Fischer (1955) und Weischer (1956) ausser an Kiefer auch an mehreren Fichtenarten Nematodenschäden nachweisen. Es handelte sich hier zum grössten Teil um *Pratylenchus-*, *Rotylenchus-*, *Ditylenchus-* und *Dorylaimus*-Arten.

Wir konnten in der schon erwähnten Baumschule in Bad-Liebenwerda in der Niederlausitz im Jahre 1955 ähnliche Beobachtungen machen, die sich auf Kiefer, Fichte und Lärche erstreckten. Einen Nematodenherd aus einem mit dreijährigen Fichten bestandenen Quartier zeigt die Abbildung. Die Ergebnisse der Boden- und Wurzeluntersuchungen sind in der Tabelle 2 zusammengestellt. Bei den Bodenuntersuchungen fällt wieder die hohe Zahl aus Lärchenquartieren auf. Dies ist jedoch auch hier darauf zurückzuführen, dass diese Proben sofort nach Entnahme ausgespült wurde, während die übrigen erst nach 8 Tagen geprüft werden konnten. Die Wurzelproben ergaben ein gleichsinniges Bild. Auch hier wiesen die Faserwurzeln nekrotische Flecke auf und waren zum grössten Teil abgestorben.

Abb. 1. Nematodenherd in einem Quartier mit dreijährigen Fichten

Tabelle II

Nadelhölzer

Bodenverhältnisse usw. wie Tabelle I.

	Stand	Nematoden in 10 ccm Boden und (in Klammern) auf 10 cm Wurzellänge *Tylenchidae*	*Dorylaimidae*	Sonstige	insgesamt
1. Larix	schlecht	46 (15)	6 (3)	7 (13)	59 (31)
	gut	3 (2)	0 (1)	11 (1)	14 (4)
2. Pinus	schlecht	16 (16)	5 (4)	3 (12)	24 (32)
	gut	0 (1)	0 (0)	1 (1)	1 (2)
3. Picea	schlecht	14 (18)	4 (2)	2 (10)	20 (30)
	gut	0 (2)	0 (0)	4 (2)	4 (4)

III. DISKUSSION

Die Gegenüberstellungen zwischen den in der gleichen Baumschule gelegenen gesunden Quartieren und den Stellen mit Müdigkeitserscheinungen zeigen Unterschiede, die klar erkennen lassen, dass den Nematoden eine Bedeutung für die in diesen Fällen beobachtete „Baumschulmüdigkeit" zugesprochen werden muss. Ob die Nematoden dabei als alleiniger Schadorganismus anzusprechen sind, sei zunächst noch offengelassen. Wir wissen, dass parasitische Nematoden Pilze und Bakterien mit sich schleppen, die selbst als Pflanzenparasiten wirksam werden können; inwieweit solche an der „Baumschulmüdigkeit" beteiligt sein können, bedarf aber noch der Aufklärung.

Zusammenfassung

Nematoden als Ursache für „Baumschulmüdigkeit" wurden für folgende Gehölze wahrscheinlich gemacht; Stein- und Kernobst, Robinia pseudacacia, Caragana arborescens, Prunus padus, Laburnum anagyroides, Sambucus rubra, Rosa pollmariana, Syringa vulgaris, Larix, Pinus, Picea. Als Parasiten wurden Tylenchiden und Dorylaimiden nachgewiesen.

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(*Nematologica* II (1957): 68-71)

PTERYGORHABDITIS, A REMARKABLE NEW GENUS OF SOIL NEMATODES

BY

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Fifteen specimens (7 female, 4 male, and 4 juvenile) of a new genus of soil nematodes were collected from damp straw in a field of winter rice near Dacca. The genus represents an interesting development in the family Rhabditidae.

Pterygorhabditis new genus

Diagnosis: Rhabditinae. Cuticle with heavy sclerotized transverse bars, arranged in longitudinal rows. Ends of adjacent bars fork and interlace to form ridges or wings. Head region retractible; lips distinct on right side only. Cephalic setae present; 4 sublateral, others asymmetrically arranged. Stoma cylindrical, telorhabdions not distinct. Oesophagus consisting of corpus, isthmus, and valved bulb. Female tail narrow and tapering, without cuticular ornamentation; anterior of tail enveloped in a loose, ventral cuticular sleeve, with heavy ornamentation. Ovaries paired, opposite, reflexed, the anterior much longer than the posterior; vulva postequatorial. Male tail short, with cuticular bursa, not enveloping the tail spike. Two pairs preanal papillae, three pairs postanal papillae, and four pairs of smaller papillae near tail tip. Phasmids distinct. Testis single. Spicules long, paired; gubernaculum short and blunt.

Type species: *Pterygorhabditis pakistanensis* new species
Cotype: in personal collection, S, 1.
Habitat: damp straw on margin of winter rice field.
Locality: Kamalapur, Dacca, East Pakistan.
Date of Collection: January, 1956.

*) Received for publication: August 8, 1956.

Pterygorhabditis pakistanensis new genus, new species

Description: Body cylindrical, tapering abruptly anteriad and posteriad from in front of anus. Cuticular ornamentation prominent, obscuring internal structures. Heavy sclerotized transverse bars just beneath the surface of the cuticle, broken into dots and strokes at the extreme anterior. Bars arranged in longitudinal rows, 10 or 12 in mature females, 8 in mature males, and 6 in third stage juveniles. Bars form a "V" at either end and interlock with adjacent bars to form a diamond-shaped pattern. Normal pattern varies in extreme anterior and posterior of body; smaller diamonds may be interspersed with larger, a "V" may be formed at end of one bar and not the adjacent, etc. Ends of bars slope upward to form ridges or wings, composed of contiguous diamonds aligned in longitudinal rows. Wings begin a short distance behind the stoma in mature specimens and at the oesophageal base in juveniles. Entire head region retractible; 3 large distinct lips on right side of body, 3 smaller indistinct lips on left side. Prominent cephalic setae, 8 μ long, one on each of the four sublateral lips; 2 shorter setae, 4 μ long, inside the long setae on right side only; many fine short setae (at least 8) on lateral and sublateral lips of left side only. Possibly an inner circle of tiny papillae on apex of each lip. Stoma 21-23μ long, without distinct cheilo- or telorhabdions. Oesophagus consisting of uniformly-expanded corpus, narrow isthmus, and terminal bulb with valvular apparatus. Nerve ring across isthmus. Excretory pore opposite posterior of nerve ring. Cervical papillae not observed.

Female: 702-850 μ; a, 11-13.1; b, 3.7-4.4; c, 8.3-9.4; V, 63.8-65%; OV_1, 22%; OV_2, 7%. Ovaries paired, opposite, reflexed, anteriormost longer. Vulva small, inconspicuous, with no lips. One fertilized egg observed in each uterus at a time, 62 $\times$ 36 μ. Tail at first conical, then narrow and uniformly tapering to a fine tip. Cuticle behind anus separated ventrally from tail tissue to form a kind of sleeve; cuticular bars extend over sleeve. Phasmids distinct.

Male: 540-675 μ; a, 10-13.5; b, 3.4-4.2; c, 10-11.6. Testis extending almost to oesophageal base. Spicules long and narrow, cephalated, 68 to 90 μ long. Gubernaculum short and blunt. Bursa extending from before anus to tail spike, bearing lightly-sclerotized striations. First 2 pairs of genital papillae slightly preanal; 3rd., 4th., and 5th. pairs just behind the anus; 6th. and 7th. pairs very faint, recognizable only by tip protruding beyond bursa margin; 8th. and 9th. small. Papillae

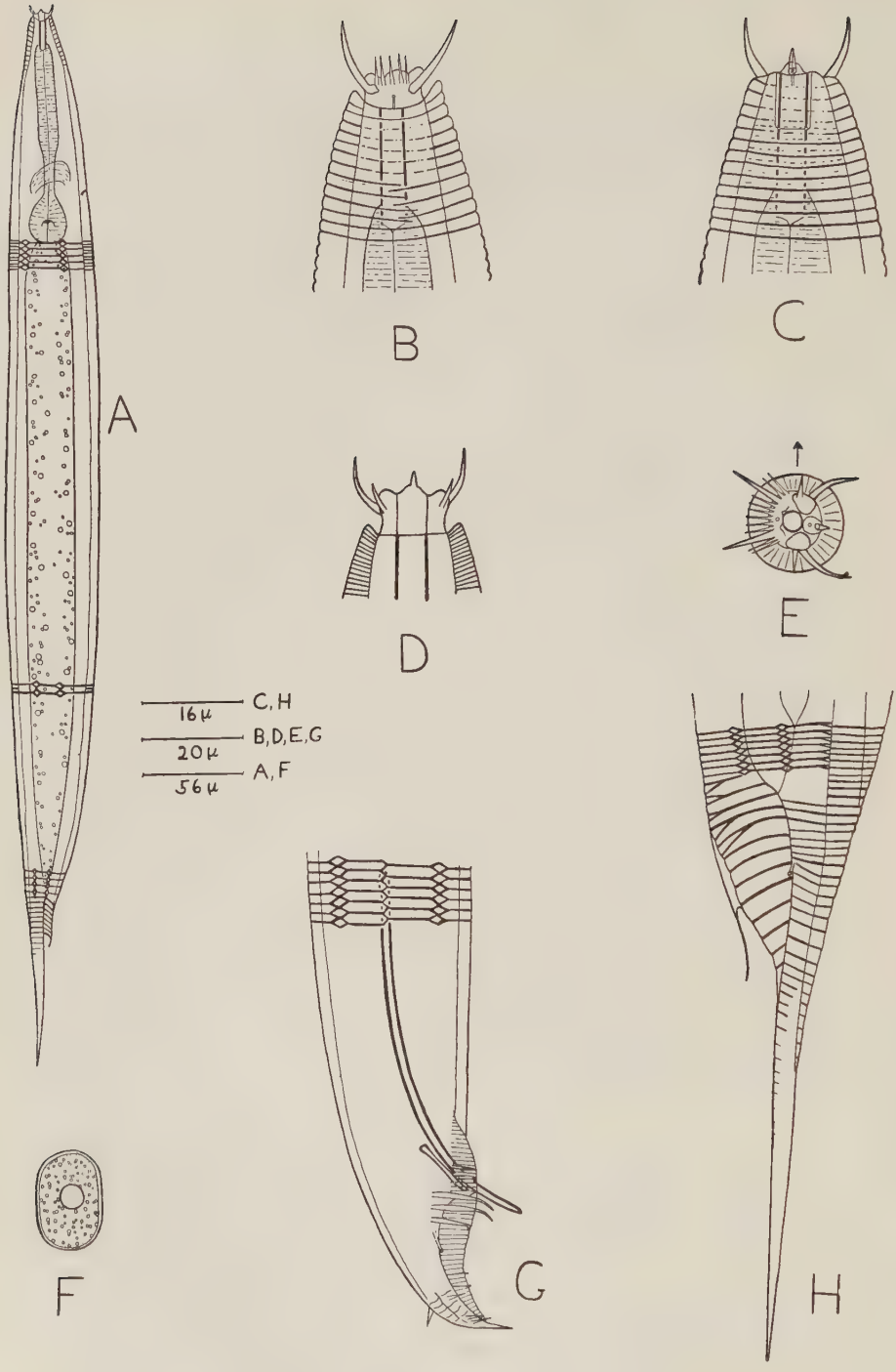

Fig. 1. *Pterygorhabditis pakistanensis* n.g., n.sp. A. Lateral view of juvenile. B. Female head from left side. C. Female head from right side, lip region retracted. D. Female head from left side in deep focus, lip region extended. E. Female head, *en face* view. F. Ovum. G. Male tail. H. Female tail.

3, 4, 6, 7, and 9 protrude beyond the bursa margin; papilla 2 turns inward and papilla 5 turns outward. Phasmids distinct, just behind papilla 5, not consisting of a modified papilla.

Discussion

At first glance *Pterygorhabditis* resembles a marine nematode of the family Desmodoridae. However, in the structure of the oesophagus and the bursal pattern it is seen to have a definite relationship to *Rhabditis*. It is remarkable for the development of the cuticular ornamentation and the asymmetrical cephalic pattern.

Acknowledgement: The writer is indebted to Dr. G. Steiner, University of Puerto Rico Agricultural Experiment Station, for many helpful suggestions.

ZUSAMMENFASSUNG

Pterygorhabditis, eine bemerkenswerte neue Gattung der Erdnematoden. In einem Feld von Winterreis wurde in feuchtem Stroh eine neue Gattung von Erdnematoden gefunden und beschrieben. Die Gattung ist hauptsächlich durch die Anwesenheit von mehreren asymmetrischen Kopfborsten und von Längsleisten charakterisiert.

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(*Nematologica* II (1957): 72-75)

A METHOD FOR DETERMINATION OF THE INFECTIVITY OF HETERODERA ROSTOCHIENSIS LARVAE *)

BY

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Studies on the infectivity of larvae of *Heterodera rostochiensis* Wollenweber were undertaken in an attempt to find a bioassay method for the comparison of populations of nematodes parasitic on plants. A reliable rapid measure of the ability of larvae to penetrate roots would be a useful tool in studies of various influences on the general health of the parasite. The experiments reported here were designed to answer the following questions:

I. How long must larvae be in contact with roots for penetration to occur?
II. What is the influence of the number of larvae present around a root on the ability of a larva to enter?
III. How does infectivity of larvae change while they are stored in water?

Procedures modified from those of Dropkin (1954) were used. Seeds of Marglobe tomato were germinated on moist filter paper in Petri dishes at room temperature. Larvae were obtained from cysts grown on tomatoes and stored in soil in the laboratory for 2 years. These cysts were extracted from the soil and placed in Syracuse watch glasses in diffusate from potted tomato plants, fresh diffusate being added each day. All the larvae used in the first and second experiments had hatched within the previous 24 hours. Seedlings with roots 2 to 3.9 cm. long were selected and transferred singly to Petri dishes containing moist filter paper. Larvae were collected with a micropipette at 15 × magnification and transferred to a drop of water on a cover glass. The cover glass was placed in the Petri dish so that the root tip entered the drop of water. Fine sand (screened through a

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sieve with 710-micron openings) was sprinkled to cover the drop of water and the root tip; then the entire root was covered with coarser sand. This sand was lightly moistened and the cover replaced on the Petri dish, which was left undisturbed for the required period. Roots were then removed, washed free of sand and prepared for examination by treatment with Flemming's fixative, followed by clearing in lactophenol. Nematodes in roots were easily seen by examination at 20 × magnification.

In the first experiment, 69 preparations (Table I) were made to find the relation between time of exposure and entry of larvae into roots. It was concluded that about 80 % of the larvae entered by the end of the second day and that little or no entry occurred after that time.

TABLE I

Infectivity of *Heterodera rostochiensis* larvae in contact with tomato roots for various periods.

Period of contact between plant and nematode	Number of seedlings	% of larvae infective (± Standard error)
24 hours	10	55 ± 6.0
48 hours	10	81 ± 2.5
72 hours	10	76 ± 2.7
96 hours	10	85 ± 1.3
120 hours	9	83 ± 3.0
144 hours	20	76 ± 3.8

The question now arose: does each larva enter a root independently, or is there some way in which one larva influences the entry of another? For example, might larvae use the entry holes made in roots by larvae that entered previously? If they do, the proportion of larvae which enter when 25 larvae at a time are placed on each root should be higher than the proportion entering when one larva is placed on each. In this series roots were fixed after exposure to infection for 48 hours. The data of Table II show that the infectivity was lower than in experiment 1, but that there was no effect of size of inoculum on infectivity. This is in agreement with results previously obtained with root-knot nematode on cucumber (Dropkin, 1954). Table II shows that 46 % of the larvae presented to the plant in groups of 25 were infective compared with 49 % of larvae presented singly.

The third experiment was designed to measure the influence of

TABLE II

Infectivity of *Heterodera rostochiensis* larvae to tomato roots under three conditions of crowding.

Number of larvae per plant	Number of plants	% of larvae infective
1	99	49
2	16	47
25	20	46

storing emerged larvae in daily changes of tap water at room temperature. Forty-four tomato seedlings (Table III) were each exposed as before to 25 larvae at various periods after emergence from the cysts. Records of the motion of larvae during storage were kept, but no relation between motility and the ability of larvae to enter roots was noted. In some instances larvae which appeared motionless were stimulated to move by changing the water in the microculture dish in which they were stored. Larvae kept for 21 days showed 76 % motility, but the vigor of motion was much less than that of freshly emerged larvae, and their infectivity was 19 % compared with 71 % for larvae stored for 7 days.

TABLE III

Infectivity of *Heterodera rostochiensis* larvae stored for various periods in daily changes of tap water.

Time between emergence of larvae and contact with plant	Number of plants	% of larvae infective (± Standard Error)	% of larvae motile
7 days	14	71 ± 5.4	76
14 days	15	24 ± 5.8	44
21 days	15	19 ± 2.3	76

Conditions affecting the penetration of parasite larvae into a suitable host may be grouped into three classes: 1) Factors affecting the larvae; 2) factors affecting the host; and 3) factors influencing penetration itself. Under 1) such variables as the strain of parasite and its previous history come to mind. The physiological condition of the plant and its genetics constitute the second class. Environmental conditions surrounding the host and parasite at the moment of invasion will obviously influence the success of penetration. In this study a step was taken in the direction of controlling some of these

many factors with the object of standardizing procedures so as to obtain comparable results from one experiment to the next. For this reason very young roots were presented to the nematodes since uniform populations of roots can easily be obtained from seeds. In some preliminary experiments, larvae of *Heterodera rostochiensis* penetrated tomato roots 3 mm. or less in length more successfully than they entered roots 3.1 to 4.8 mm. long. By selecting roots of uniform length, produced by seeds of a single variety germinated under uniform conditions, one should be able to minimize the variability affecting the host plant. Similarly, one may use fresh larvae which have all emerged during a short time. The cover glass technique, with a drop of water of uniform size, maintained at constant temperature, offers the possibility of controlling environmental conditions of penetration. In this way it should be possible to determine infectivity of nematodes from a single population under a variety of treatments and to compare separate populations with regard to this characteristic.

SUMMARY

A bioassay system for the measurement of the ability of nematode larvae to enter plant roots is likely to be useful in many ways. Simple procedure in which larval entry is complete within 48 hours is described. Exposures to single larvae yield results comparable to exposures to groups of 25 larvae. Infectivity declines as larvae are stored in water. No correlation between motility and infectivity of stored larvae was found.

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STUDIES ON ROOT-KNOT NEMATODES OF THE GENUS MELOIDOGYNE IN THE GOLD COAST

PART I.

COMPARATIVE DEVELOPMENT ON SUSCEPTIBLE AND RESISTANT HOST SPECIES [1])

BY

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Factors influencing the host-parasite relationship have been shown to be environment, host suitability, and specificity of the eelworm population (Godfrey 1931, Godfrey and Oliveira 1932, Tyler 1933). Barrons (1939) suggested that larvae enter the roots of resistant plants as readily as they do those of susceptible plants, but fail to develop in the former. Sasser and Taylor (1952) showed that, in addition to normal entry and development of root-knot larvae, there is also (i) failure to enter roots, (ii) entry of reduced numbers with little or no development, (iii) entry of large numbers with varying degrees of development ranging from none to a few individuals reaching maturity, and concluded that resistance was not of the same nature in all plants. Macbeth and Taylor (1944) have shown that *Crotalaria spectabilis* Roth reduced the severity of root-knot on tobacco grown subsequently. Stevenson and Jones (1953) list root-knot resistant varieties of cowpea, soybeans, and peppers.

The development of a root-knot nematode population on a number of susceptible and resistant host plants has been studied in the Gold Coast. Varieties of maize and cowpea, and some common legumes, have been examined for resistance to root-knot eelworm.

METHODS

Plants were raised from seed sown in sterile loam soil in 3½" pots; seedlings were thinned to leave 2 plants per pot, and the soil around the roots was inoculated with a suspension in distilled water of newly hatched larvae. Each plant received a 'dose' containing 500 larvae

1) Received for publication: September 7, 1956.

2) Now at Jealott's Hill Research Station, Bracknell, England.

(i.e. 1,000 per pot). The nematode population used for the inoculum was a culture of larvae of *Meloidogyne* sp. On the basis of host-specificity tests (Sasser 1954) it is suggested that this nematode is *Meloidogyne incognita* var. *acrita*. It is parasitic on red pepper and maize, but not on groundnut; it is not, or only sparingly, parasitic on cotton, but Sasser and Nusbaum (1955) suggest that this species has strains or races which differ in their ability to attack certain crops, of which cotton is one. Measurements and study of the perineal pattern (Edwards, unpublished) has so far not made confident identification possible. Egg-masses were collected from a single tomato plant, washed and placed in distilled water in petri dishes. The water containing newly hatched larvae was drawn off each day with a pipette and replaced with distilled water. In this way it was possible to use larvae all hatched during the same 24-hour period. Plants were examined at 2-day intervals, the roots being washed and stained by the acid fuchsin/lactophenol technique (Goodey 1949). Observations were made on (i) host reaction, (ii) development of eelworm larvae within the root (using the grouping of *Meloidogyne* stages of development suggested by Christie 1946), (iii) production of the gelatinous matrix, oviposition, first viable eggs, egg-counts and percentage of viable eggs.

Two root-systems (i.e. the contents of one pot) were washed, stained and cleared, for each of the species of plant. Whole roots in lactophenol were examined carefully under a lowpower dissecting microscope first, then selected pieces were dissected out; when counts of nematodes in different growth categories were required, these selected pieces were placed between two glass slides and gently pressed whilst watching through the microscope. In this way all nematodes in the tissue were seen including some which failed to take up the stain adequately. Soil temperatures during the 33-day period of observation varied from 26°-31° C.

OBSERVATIONS

Table 1 illustrates the varied response of different plants to the parasite.

Although the inoculation consisted of larvae hatched within the same 24-hour period, considerable variation occurred in the life-cycles of individuals. Larvae were deposited on inoculation at varying distance from a root, roots and parts of root within the same root-system are known to be differentially attractive (Linford 1939) or even repel-

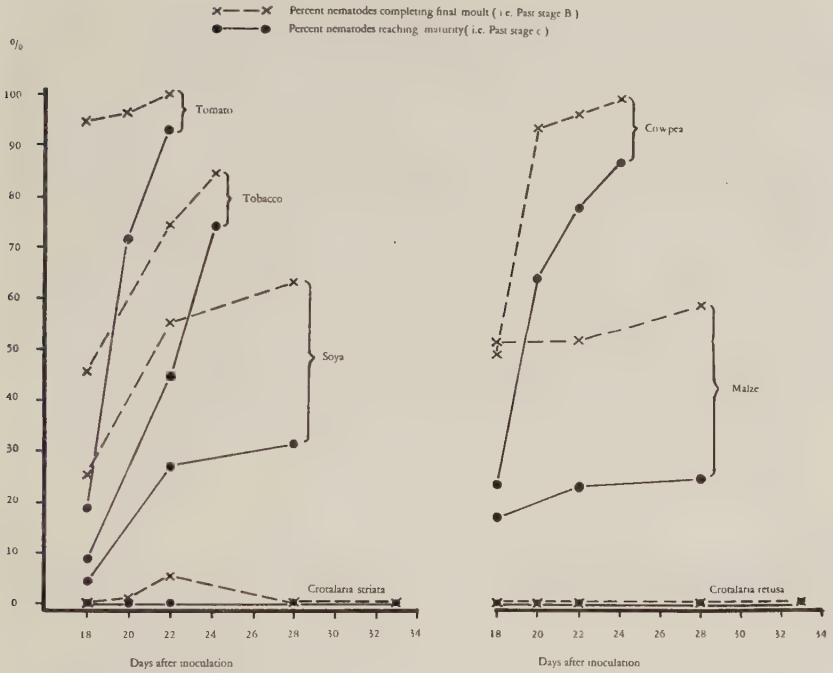
TABLE I

Reaction of certain plant roots to nematode invasion

Host	Time to appearance of first swelling in days	Form of swelling	Nematodes contained in each swelling	Subsequent development over 30-day period
Zea mays L. Maize	10	minute spherical	1-3	Little or no increase in size of initial swellings
Lycopersicon esculentum Mill. Tomato	5	large sub-spherical	many	Rapid increase in size of swellings
Glycine max Merr. Soya	8	elongated but little thicker than the root	many	Slow increase in size of swellings
Nicotiana tabacum L. Tobacco	8	elongated	many	Rapid increase in size of swellings
Vigna sinensis Endl. Cowpea	5	large sub-spherical	many	In many cases, a proliferation of side roots
Crotalaria retusa L.	8	clubbing of root tips	1-3	Roots became wrinkled and necrotic, short side roots formed also became clubbed
Crotalaria striata D.C.	8	slight swelling initially only	4-5	No further development of initial swelling

lent (WIESER 1955); upon entering the roots, individuals may develop at different rates depending on the particular host/parasite relationship obtaining in that region of the root. For this reason in addition to recording the shortest time after inoculation for individual larvae to reach various stages of growth (Tabel II), the proportion of larvae reaching certain stages at given times was noted (Fig. 1).

In terms of host suitability, these criteria show basically the same picture; tomato is the most suitable host, followed by cowpea, tobacco, soya and maize, while the *Crotalaria* spp. although invaded, can scarcely be considered as true hosts. Table II, however, shows relatively small differences, for example, first individuals to complete the final moult on maize, did so only 2 days after their counterparts on tomato; viable eggs were produced on maize at 33 days, as compared with 28 days on tomato. Fig. 1 shows that differences in host suitability are in fact much greater as reflected by the time-development curves.



Fig, 1. Development curves for *Meloidogyne* Sp. on seven plants

Table II

Comparative development of root-knot eelworm on various hosts, as indicated by most advanced individuals

Host plant	Days after inoculation, first individuals recorded in stage: * A	B	C	D	E	Matrix produced	Eggs laid	Viable eggs
Zea mays L. Maize	4	12	16	18	26	26	28	33
Lycopersicon esculentum Mill. Tomato	2	8	14	16	20	20	20	28
Glycine max Merr. Soya	4	10	16	18	22	22	24	33
Nicotiana tabacum L. Tobacco	6	12	16	18	24	24	24	33
Vigna sinensis Endl, Cowpea	2	8	14	16	20	20	22	28
Crotalaria retusa. L.	6	20	—	—	—	—	—	—
Crotalaria striata D. C.	6	18	20	—	—	—	—	—

* The stages are those of Christie (1946)

Table II shows that first individuals to produce the gelatinous matrix did so at 20 days after inoculation on tomato and cowpea, 22 days on soya, 24 days on tobacco, and 26 days on maize: (Egg-laying females, or CHRISTIE's Group 'E' were recorded as such from the time the matrix formed). Eggs were laid from less than 1, to 2 days after the matrix appeared and egg-masses were formed on the outside of the rootlets of all host plants (see Discussion). Limited egg-counts and viability determinations during the early stages of egg-laying (Table III) showed no striking differences in the rate of egg-production.

When examining egg-masses on tomato and on cowpea a number of "double" eggs were observed. These were markedly elongated, (in an egg-mass where 'normal' eggs measured 82-92 $\mu \times$ 42-49 μ, 'double' eggs were 130-136 $\mu \times$ 42-46 μ) and sometimes occurred in appreciable numbers, in one small eggmass on cowpea there were 10 'double' eggs. None of those observed was viable.

TABLE III

Egg-production during first few days of egg-laying — means of 10 observations

Host plant	Days after inoculation	Mean number of eggs per female	Mean % eggs containing larvae
Zea mays L. Maize	26	0	0
	28	23	0
	33	120	4
Lycopersicon esculentum Mill. Tomato	18	0	0
	20	8	0
	22	40	0
	24	146	0
	28	212	14
	33	435	32
Glycine max Merr. Soya	22	0	0
	24	5	0
	28	33	0
	33	172	16
Nicotiana tabacum L. Tobacco	22	0	0
	24	22	0
	28	88	0
	33	210	17
Vigna sinensis Endl. Cowpea	20	0	0
	22	28	0
	24	70	0
	28	166	23

In order to ascertain whether any of the viable larvae seen were capable of invading plant tissues, small tomato plants were planted among the roots of the infested tomato, soya, maize, cowpea and tobacco plants 36 days after initial inoculation. The small tomato plants were examined 4 days later when larvae were found in all roots except maize. Largest numbers were found in tomato after cowpea — more than 2,000 per plant. Fewest were found after tobacco, but even so each root tip contained 6-10 larvae. No larvae were found in tomato after maize. In the subsequent experiment, penetration of fresh tomato roots by second-generation larvae occurred within 30 days and four generations of root-knot eelworm were obtained in as many months. Thus, 10-12 consecutive generations per year can occur under favourable conditions.

Resistant plants

Groundnut, (*Arachis hypogaea* L.) is completely resistant to root-knot eelworm in the Gold Coast. In tests on a number of plants using eelworms from different populations no larva was observed to gain entry into the roots. *Crotalaria striata* D.C. and *C. retusa* L. were also resistant, larvae entering the roots but failing to develop to maturity (see Fig. 1). Sun hemp (*C. juncea* L.) and *C. usaramoensis* Bak., Jack bean (*Canavallia ensiformis* D.C.) and Velvet bean (*Stizolobium deeringianum* Bort), developed root-knots after 3 weeks, and egg-

TABLE IV

Differential susceptibility to Eelworm in maize varieties

Variety	Origin	No. of larvae in roots after 24 hours. Mean of 4 plants	No. of larvae in roots after 3 days. Mean of 4 plants	No. of egg-masses on roots after 32 days. Mean of 2 plants
White Tuxpan	Introduced	0	2	46
Bulon corn	„	0	9	51
Mexican 1	„	0	0	68
Trinidad	„	1	16	30
Sicaragua	„	0	6	—
Hybrid corn *)	„	0	0	9
Kawena	Local	5	222	144
Kawenzia A	„	2	146	125
Kawempliga	„	0	0	65
Kawebariba	„	0	1	61
Keta 60-day	„	0	9	49

*) Single-cross hybrid between a Mexican line and an American sweet corn.

masses were observed after 6 weeks. Observations on varieties of maize (*Zea mays* L.), grown in sterile soil and inoculated with a larval suspension, are given in Table IV. A hybrid corn showed a high degree of resistance.

Two varieties of cowpea (*Vigna sinensis* Endl.) which yielded well on eelworm-infested land at Ibadan (Vernon, in litt.) were compared with the local creeping cowpea in a small plot experiment. Results (Table V) showed one variety, Machakos, to be highly resistant.

Table V

Differential susceptibility to Eelworm in cowpea varieties

Variety	Plant growth marks. Scale 1-10; after 20 days	Mean No. of knots per root; after 34 days	Mean root-knot grading. Scale 1-10; after 65 days
Local creeping	5.7	188.0	5.3 ± 0.37
New Era (ex U.S.A.) obtained from Ibadan	9.0	128.7	6.7 ± 0.44
Machakos (ex Kenya) obtained from Ibadan	7.7	0.7	0.3 ± 0.08

Machakos was virtually free from knots. New Era was apparently more susceptible than the local variety, but this is due to a low figure on one plot only of the local variety, on which plants had begun to die down. When these plants were lifted many knotted roots may have broken off and remained in the soil.

Discussion

The completion of the root-knot eelworm life-cycle in 28 days on tomato in the experiments described above, at soil temperatures of 26-31° C, may be compared with work by Tarjan (1952). At a soil temperature of 20.4° C, Tarjan showed that "penetration of root-tips by second generation larvae of *Meloidogyne incognita* occurred at 57 days after inoculation, of *M. incognita* var. *acrita* 59 days, and of the other 3 spp. investigated 63 days after inoculation." This followed egg-production at 37-39 days.

A contributory factor to the rapidity with which a root-knot infestation builds up under Gold Coast conditions is the formation of egg-masses outside the root, discharging larvae directly into the surrounding soil.

Godfrey (1931) and Godfrey & Oliveira (1932) reported that egg-masses on cowpea frequently break through to the exterior of the

root but that on pineapple, and indeed as a normal condition on both plants, egg-masses were formed in the interior of the gall. GODFREY suggested that external egg-masses were formed only at high soil temperatures.

In the Gold Coast egg-masses are normally extruded through the epidermis of the root on all host plants, but where infestation is heavy, and where the host reaction is pronounced e.g. on tomato, and *Cucurbitaceae,* both internal and external eggmasses are found. This may be important in root-knot control since external egg-masses are likely to be much less resistant than egg-masses protected by gall tissue.

SUMMARY

The development of the root-knot nematode on a number of hosts in the Gold Coast was studied, and the host/parasite relationships compared.

The life-cycle of the most advanced individuals on tomato and cowpea was completed in 28 days, at soil temperatures of 26-31° C; on maize, soya and tobacco the life cycle occupied 33 days, but individual variation was considerable and the "average" life-cycle differed much more from host to host. On two species of *Crotalaria* the life cycle was never completed, larvae dying before, or shortly after, completing the final moult.

The rate of egg-production did not differ markedly on different hosts during the limited period under observation, but total egg-production was not studied.

A variety of cowpea, Machakos, and a hybrid maize were shown to be resistant to root-knot attack; two species of *Crotalaria* were highly resistant, but Jack bean and Velvet bean and two other *Crotalaria* spp. were not resistant.

ACKNOWLEDGEMENTS

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(*Nematologica* II (1957): 85-90)

TROPHOTYLENCHULUS AND TROPHONEMA, TWO NEW GENERA OF TYLENCHULIDAE N. FAM. (NEMATODA) [1]

BY

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In 1952 RASKI and SHER described a new genus and species, *Sphaeronema californicum,* from the roots of Umbellularia californica Nutt. in Marin County, California and from the roots of Arctostaphylos sp., El Dorado County, California. A new subfamily, the Sphaeronematinae, was also established and placed in the family Criconematidae. Later (1956) RASKI described a new species, *Sphaeronema arenarium,* from the roots of Juncus leseurii Boland., collected in Marin County, California.

Recently (1955) the author collected additional nematodes from the roots of Quercus falcata Michx., near Winter Haven, Florida. These also appear to be of the same subfamily but to represent a new genus, *Trophotylenchulus* n. g. this article, definitely linking *Sphaeronema* with *Tylenchulus* Cobb, 1913. The collection of these new specimens also indicates that the females of *S. arenarium* should be placed in a different genus than *S. californicum.* Therefore, *Trophonema* n. g. is proposed to receive *Trophonema arenarium* n. comb.

SKARBILOVICH (1947) placed the subfamily Tylenchulinae [2] in the family Heteroderidae. However, the above four genera appear to represent a distinct group of nematodes that do not belong in Criconematidae, Heteroderidae or Tylenchidae. In order to show their proper relationship these genera are transferred to Tylenchulidae n. fam. which is characterized as follows: Stylet well developed but not greatly elongated in larvae and females; head lightly sclerotized; esophagus consisting of well defined procorpus, metacorpus, isthmus and glandular region; metacorpus greatly enlarged and elongated; esophageal glands enclosed; cuticle finely annulated; females saccate

1) Received for publication: September 17, 1956.

2) This category was originally designated as Tylenchululinae by SKARBILOVICH but the addition of *inae* to the root of the type genus, *Tylenchulus,* should correctly read Tylenchulinae.

with one ovary; males without caudal alae (Parasites of plant roots external, semi-internal or internal).

Subfamily Tylenchulinae Skarbilovich, 1947. Excretory pore located posterior to normal position; male stylet degenerate (*Tylenchulus, Trophotylenchulus*).

Subfamily Sphaeronematinae Raski and Sher, 1952. Excretory pore normal, near nerve ring; male stylet lacking (*Trophonema, Sphaeronema*).

Trophonema n. g.

Diagnosis. — Subfamily Sphaeronematinae: Considerable sexual dimorphism present; females spiral, thickened; males slender; female esophagus tylenchoid, median bulb conspicuous; stylet well developed; anus indicated but apparently non-functional; female tail arcuate, thick; one ovary anterior to vulva; oviparous, one egg at a time in uterus deposited in a mucoid mass surrounding vulva; body greatly reduced in diameter posterior to vulva; male tail elongate conoid with sharply pointed terminus; spicules arcuate, setaceous; caudal alae absent.

The name *Trophonema* is derived from τροφός, one who feeds, and νῆμα, thread, and is neuter in gender.

Type species. — *Trophonema arenarium* (Raski, 1956) n. comb.

Synonym: Sphaeronema arenarium Raski, 1956.

The genus *Trophonema* differs from the genus *Sphaeronema* primarily in the shape of the females which in *Sphaeronema* are greatly modified into spherical adults with a coarse, reticulate cuticular pattern and a prominently protruding vulva. *Trophonema* is further differentiated from *Trophotylenchulus* under the diagnosis of that genus.

Trophotylenchulus n. g.

Diagnosis. — Subfamily Tylenchulinae: Considerable sexual dimorphism present; females spiral, thickened; males slender; lip region with distinct circumoral elevation in females and larvae; esophagus tylenchoid, median bulb conspicuous; stylet well developed; anus indicated but apparently non-functional; excretory pore far posterior to esophagus (33-44 % from anterior end of body), even more posterior in larvae; female tail arcuate, thick; one ovary anterior to vulva; oviparous, one egg at a time in uterus, deposited in a mucoid mass surrounding vulva; body greatly reduced in diameter posterior to

vulva; male tail elongate, conoid with bluntly rounded terminus; spicules arcuate, setaceous, with distinct bend near distal end; caudal alae absent.

The name *Trophotylenchulus* is derived from τροφός, one who feeds, τύλος, a knot or callus, and ἔγχος, spear, to which is added the Latin diminutive suffix *-ulus* making the name masculine in gender.

Type species. — *Trophotylenchulus floridensis* n. sp.

The genus *Trophotylenchulus* differs from its most closely related genus, *Tylenchulus* Cobb, 1913, primarily in the distinct modification of the lip region in *Trophotylenchulus* with a circumoral elevation present in the larvae and females which does not occur in *Tylenchulus*. In addition the position of the excretory pore is further posterior in all stages of the latter genus being very close to the vulva in the adult female. The new genus also differs from *Trophonema* in the modification of the lip region and the more posterior situation of the excretory pore, really being intermediate in this respect between *Trophonema* and *Tylenchulus*.

Trophotylenchulus floridensis n. sp. (Fig. 1, a-g)

1 ♀ : .44 mm. a = 5.3 b = 2.4 c = 18.6
V = $^{57}80^{1.5}$ Stylet = 15 μ

7 ♂♂ : .41-.58 mm. a = 32-41 b = ? c = 7.6-9.9
T = 27-41% Stylet = 10-12 μ

Female (*Holotype*). — .44 mm. a = 5.3 b = 2.4 c = 18.6 V = $^{57}80^{1.5}$. Body swollen near vulva, tapering anteriorly, greatly reduced in diameter posterior to vulva; body annules 2.5 μ near middle of body; lip region with distinct circumoral elevation, without definite annulation; labial framework lightly sclerotized; stylet 15 μ long, prorhabdion approximately 50% of stylet length, knobs 2 μ by 3 μ, rounded; dorsal esophageal gland orifice 7 μ behind stylet base; lining of corpus unusually thick; bulb elongate, massive; excretory pore posterior to espohagus, 123 μ (33% of body length) from anterior end; spermatheca not observed but found present in other paratype specimens; ovary well developed, extending to base of median bulb and reflexed twice; posterior uterine branch very short or absent; anal opening obscure, marked by slight elevation of cuticle, probably non-functional; tail arcuate, terminus bluntly rounded at end; lateral field not observed.

Male (*Allotype*). — .44 mm. a = 31.6 b = ? c = 9.2 T = 31%. Body slender, cylindroid, tapering anteriorly and posteriorly; annules

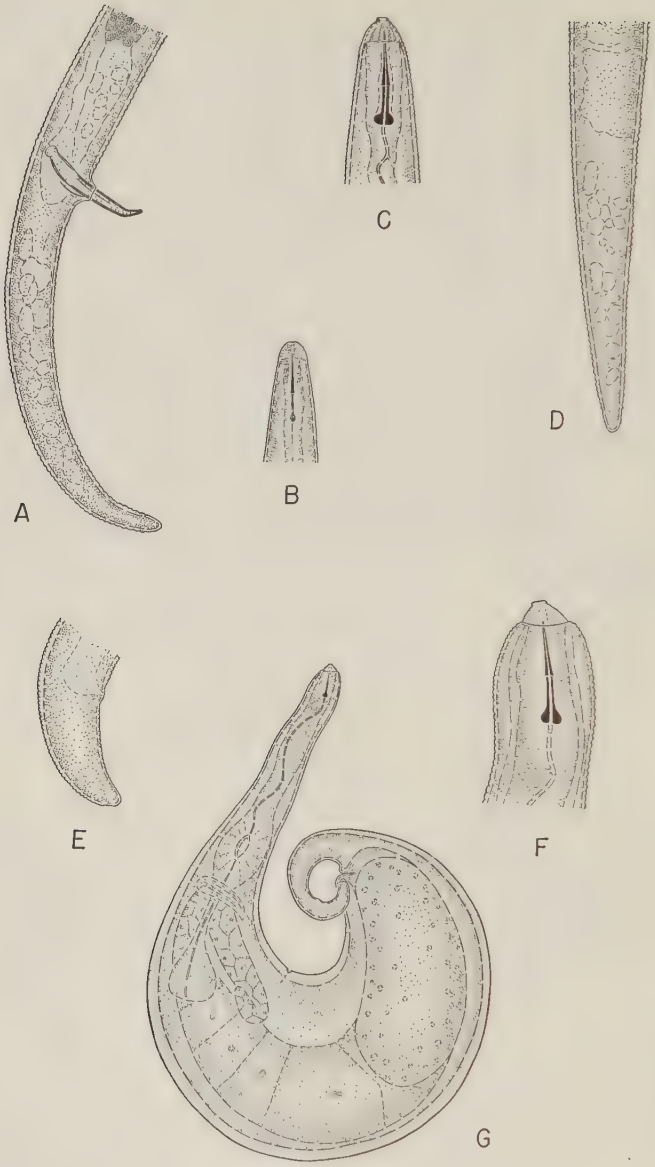

Fig. 1. — *Trophotylenchulus floridensis*. A. — Male tail, × 1000; B — Head of male, × 1000; C — Head of larva, × 1000; D — Tail of larva, × 1000; E — Tail of female, × 1000; F — Head of female, × 1000; G — Female, × 330.

1.1 μ wide; lip region continuous with body contour, without definite annulation; labial framework very slightly sclerotized; stylet 11.5 μ long, weakly developed, knobs represented only by slight swellings; excretory pore 175 μ (39.5% of body length) from anterior end of body; hemizonid approximately two body annules wide, immediately posterior to nerve ring; esophagus degenerate; spicule slightly curved, approximately 20 μ long, with distinct bend near distal end; gubernaculum simple, slightly curved, 5 μ long; spicule sheath conspicuous; caudal alae absent; tail conoid, ventrally curved, with bluntly rounded terminus.

Larva. — .41-.48 mm. a = 27-31 b = 2.6-3.5 c = ?.

Body slender, tapering anteriorly and posteriorly; annulation 1.2-1.3 μ wide; lateral field not observed; lip region continuous with body contour, without definite annulation but with distinctive circumoral elevation similar to that of female; labial framework lightly sclerotized; stylet 14-15 μ long, knobs 2 μ by 3 μ, rounded; dorsal gland orifice 4-4.5 μ behind spear base; excretory pore 164-194 μ (38-44% of body length) from anterior end; hemizonid approximately 2 body annules wide, 92-102 μ from anterior end; nerve ring immediately anterior to hemizonid; anus obscure, probably non-functional; tail elongate conoid with blunt terminus; genital primordium 140 μ from tip of tail.

Holotype. — Female collected Nov. 20, 1955 by D. J. Raski. Catalogue number 99 University of California Nematode Survey Collection, Berkeley.

Allotype. — Male, same data as holotype. Catalogue number 100 University of California Nematode Survey Collection, Berkeley.

Paratypes. — 5 females, 5 males, same data as holotype, deposited in University of California Nematode Survey Collection, Berkeley; 6 females, 6 males, same data as holotype, deposited in State Plant Board of Florida, Nematology Collection.

Type host. — Quercus falcata Michx., roots.

Type locality. — Lake Spirit Road, approximately ¼ mile south of Auburndale-Eloise Highway, near Winter Haven, Florida.

Other hosts. — Roots of Magnolia sp. near Lake Alfred, Florida collected by Dr E. P. Ducharme of the Citrus Experiment Station, Lake Alfred, Florida. Also roots of persimmon, Diospyros sp., collected by James West on the R. S. King property, Lake County, Florida.

Discussion. — Female specimens on Quercus falcata Michx. were found to occur individually in small, darkened galls composed of host root material apparently two cells or more in thickness. These galls were readily dislodged. The females were partially imbedded in the root and the heads were generally broken off during dissection making it difficult to recover entire specimens.

ZUSAMMENFASSUNG

Die Beschreibung von *Trophotylenchulus floridensis* n.g. n.sp. aus den Wurzeln von Quercus falcata Michx. bestätigt endgültig die Verwandtschaft von *Sphaeronema* Raski und Sher, 1952 mit *Tylenchulus* Cobb, 1913. Es wird vorgeschlagen, für *Sphaeronema arenarium* Raski den Namen *Trophonema arenarium* n. comb. anzunehmen. Die vier Gattungen werden zur neuen Familie der Tylenchulidae gestellt, folgenderweise: Unterfamilie Tylenchulinae Skarbilovich, 1947 (*Tylenchulus, Trophotylenchulus*) und Unterfamilie Sphaeronematinae Raski and Sher, 1952 (*Trophonema, Sphaeronema*).

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(*Nematologica* II (1957): 91-93)

PATHOGENICITY OF AN ECTOPARASITIC NEMATODE, XIPHINEMA DIVERSICAUDATUM, ON STRAWBERRIES [1])

BY

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U.S.D.A., Agric. Research Service, Hortic. Crops Branch, Beltsville, Maryland, and New York St. Agric. Exp. Sta., Geneva, N.Y., respectively [2])

Nematodes of the genus *Xiphinema* have been found associated with both cultivated and wild strawberrries (BOSHER, 1954; BROOKS, 1952; GOHEEN and BRAUN, 1956). In view of recent greenhouse experiments (SCHINDLER, 1957) demonstrating the parasitism and pathogenicity of one species of dagger nematodes, *X. diversicaudatum* (Micoletzky, 1927) Thorne, 1939 on rose and fig plants, it appeared advisable to determine its effects on strawberry plants.

MATERIALS AND METHODS

In general, the procedures used were similar to those described for experiments on the effects of *X. diversicaudatum* on rose and fig plants (SCHINDLER, 1957). The nematodes were obtained from populations of *X. diversicaudatum* established and maintained on rose plants in pots in the greenhouse. The screenings obtained by processing soil from these pots were washed into beakers and dilution counts were made. Then, after standing for 1 hour, the upper $^1/_3$ to $^1/_2$ of the water from which the nematodes had settled was decanted. Twenty strawberry plants, $^3/_4$ to 1 inch in height, grown from seed obtained from a cross of two commercial varieties, were transplanted into steam-sterilized soil in 5-inch clay pots. Inoculation consisted of pouring either water containing the specimens of *X. diversicaudatum* or the decanted water onto the roots of plants at the time of transplanting.

The pots were divided into 4 groups of 5 pots each by random selection. Each group received 1 of the following treatments:

1) Received for publication: 22 September, 1956.

2) Temporary appointment as Nematologist, Section of Nematology, Hort. Crops Res. Br., U.S.D.A., while on sabbatic leave from N.Y. State Agric. Exp. Sta. July 1, 1955 through June 30, 1956.

(1) No treatment.
(2) Approximately 100 ml. of the decanted water.
(3) Approximately 530 nematodes.
(4) Approximately 2,600 nematodes.

Pots were arranged in a randomized-block design on the greenhouse bench. To eliminate the possibility of cross contamination they were separated by 12 inches of space and each pot was set in a saucer and watered from below during the course of the experiment.

After 12 weeks the plants were removed from the pots and their roots were washed clean of soil. The results of the treatments were determined by (1) recording the numbers of runners and runner plants produced by each plant, and (2) separately weighting the tops, including runners and runner plants, and the roots of each plant.

RESULTS

From the time of planting to the termination of the experiment all plants in treatment 4 made little or no increase in growth of either above-ground parts or roots, having an average total weight of only 1.4 gms. at the end of the experiment. In contrast, plants in treatments 1, 2, and 3 had average total weights of 19.4, 18.6, and 15.8 gms., respectively. The mean top weight of the plants in treatment 4 was significantly less, at the 1% level, than that of all other treatments (Table 1). The mean root weight of plants in treatment 4 was significantly less, at the 1% level, than in treatments 1 or 2. There was also a highly significant difference in the mean root weights in treatments 1 and 3.

TABLE 1

Effects of *Xiphinema diversicaudatum* on the growth of strawberry seedlings.

Treatment	Mean top wgt. (gm.)	Mean root wgt. (gm.)
(1) Control	13.3	6.1
(2) Supernatant water	13.7	4.9
(3) Approximately 530 nematodes	13.0	2.8
(4) Approximately 2,600 nematodes	0.7	0.7
L.S.D. .05	7.3	2.3
L.S.D. .01	10.7	3.3

Figure 1. Strawberry plants showing the effects of the ectoparasitic nematode, *Xiphinema diversicaudatum*. Left to right: No treatment; Approximately 100 ml. of the decanted water; Approximately 530 nematodes; and approximately 2,600 nematodes.

No runners or runner plants were produced by any of the plants in treatment 4. Plants in treatments 1, 2, and 3 produced an average of 3.4, 3.6, and 3.6 runners and 7.6, 8.0, and 8.8 runner plants, respectively. The differences in mean root weights in treatments 1, 2, and 3 are not reflected in the numbers of runners or runner plants.

The roots of all plants in treatments 1 and 2 were abundant and healthy. Roots were somewhat less abundant on the plants in treatment 3 (Figure 1), a few of the older roots were dead, and several roots near the ground line were galled. On the plants in treatment 4 there were only a few living roots and these were severely galled.

DISCUSSION

From the results of this experiment it is evident that *X. diversicaudatum* is a pathogen of strawberries, and when present in sufficient numbers may seriously inhibit their growth. The decrease in root growth of plants in treatment 3, under greenhouse conditions, indicates that nematodes at lower concentrations may significantly affect root growth without affecting top weight and runner-plant production.

This effect is not fully understood. Since an intermediate series of inoculations was not included it is not known whether there would be a gradual or an abrupt change in the numbers of runners and runner plants produced. If the change were abrupt it would seem more likely that it was due to the actions of either a growth regulator or a growth inhibitor rather than to other causes.

Under unfavorable conditions or after a longer period, the decrease in root growth might adversely affect top growth.

Since the differences obtained with heterogeneous strawberry seedlings were statistically significant, the authors feel that the findings of this experiment would be applicable to many commercial varieties.

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BOOK REVIEW

RÜHM, W. 1956. Parasitologische Schriftenreihe Heft 6: Die Nematoden der Ipiden, Gustav Fischer, Jena, 437 Pages, 145 Fig. Brosch: DM 35.

This monograph summarizes RÜHM's investigations on nematodes inhabiting Ipidae (Coleoptera). It contains a critical analysis of the existing literature and presents much new information. RÜHM studied the nematode fauna of 59 species of Ipidae, listing in the systematic part of his monograph 179 species of which 78 are new to science. In the first part of the book the author pays special attention to the macro- and ecoclima of the host species and to the microclimazones of the trees, in which beetles and nematodes come together, the various niches which they occupy, the conditions of life therein and the influence which these conditions exert on both beetles and nematodes. The habitats in question afford peculiar surroundings for the nematodes, their 'edaphic' conditions being largely dependent on the quality of the sawdust and frass in the tunnels made by Ipidae. RÜHM studied the complete life cycles, the mode of reproduction of the nematodes concerned and their method of invasion of the beetles. He also describes the interesting sexual relations found in many forms, (e.g. coryogamy) and the phases of specific adaptation in relation to the Ipid hosts. The succession of nematodes in the tunnels made by the beetles was also studied and is dependent on the time of invasion of the nematode into the species of insect concerned. An important rôle is played by the so-called "Dauerlarven", the behaviour of which is described in detail.

Rühm's discussion on the phylogenetic relationships between nematodes and beetles is of interest. He thinks, in accordance with EICHLER, FUCHS and SZIDAT, that the parasitic Nematodes of Ipidae reflect the phylogenetical history of their hosts, in other words that the evolutionary phase of the parasite tells us something about the relative age of its host species. Primary hosts, for example, harbour primary parasites.

The second part of the book deals with the systematics of the nematodes living in Ipidae. They belong to the Allantonematidae with the genera *Allantonema, Parasitylenchus* with its new subgenera *Sulphuretylenchus* and *Metaparatylenchus*; to the Contortotylenchidae new fam. with the new genera *Contortotylenchus, Polymorphotylenchus* with the new subgenera *Polymorphotylenchus,* and *Thylakotylenchus*; and to the Neotylenchidae with the genera *Neotylenchus, Deladenus, Styctilus* and *Nothotylenchus* brought to the subfamilies Neotylenchinae, Paurodontinae and Nothotylenchinae. The Tylenchidae follow with *Ditylenchus, Sychnotylenchus* (n. gen.) and *Tylenchus*. In the Aphelenchoidea we find the Sphaerulariidae, the Aphelenchidae with its subfamily Aphelenchinae embracing the genera *Cryptaphelenchus, Aphelenchoides* with its subgenera *Aphelenchoides* and *Bursaphelenchus,* as well as the new genus *Tylaphelenchus,* and the new subfamily Parasitaphelenchinae with *Ectaphelenchus* and *Parasitaphelenchus*. The Rhabditoidea come next comprising the Cephalobidae, Rhabditidae, and Diplogasteridae. The book ends with a short summary of contents and an extensive bibliography. It is illustrated with excellent figures and will be valuable for every nematologist endeavouring to penetrate into this puzzling field in which it opens new perspectives.

J. H. SCHUURMANS STEKHOVEN

NEMATOLOGICAL NEWS

GENERAL

The 4th International Congress of Crop Protection will be held in Hamburg/ Germany from the 8th to the 15th of September 1957. Among other subjects nematode pests of crops will be discussed. For further information please ask the "Biologische Bundesanstalt für Land- und Forstwirtschaft, B r a u n s c h w e i g (Germany), Messeweg 11-12."

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(*Nematologica* II (1957) : 97-107)

EFFECTS OF SOME FIELD MANAGEMENT SYSTEMS ON ROOT KNOT OF TOMATO *)

BY

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I. INTRODUCTION

The importance of root knot nematode as a parasite of field tomatoes in the Red Cliffs Irrigation area, Victoria, has been demonstrated by BROCK & GILES (1949) and GILES & BROCK (1952), who investigated certain aspects of the control of root knot by soil fumigation. Because of the expense involved, soil fumigation, though effective, is not always practicable. A trial to determine the effects of field management systems on root knot nematode population was commenced in 1951, using the actual plots laid down by BROCK & GILES.

The root knot nematode concerned had been identified as *Heterodera marioni* (Cornu) Goodey some years before. With the re-classification of the root knot nematodes by CHITWOOD (1949) into several distinct species, re-examination of the nematodes at Red Cliffs became desirable. Dr. J. W. SEINHORST, of Wageningen, has identified the nematode as *Meloidogyne javanica* (Treub) Chitwood. Intersexes were found to be common among the males, as recorded by CHITWOOD. All perineal patterns cut from nematodes in tomato roots from the plots during the course of the experiments have been of the *M. javanica* type.

These nematodes have consistently failed to produce galls on *Lycopersicon peruvianum* in the field, thereby differing from the *M. javanica* population studied by SASSER (1954). Peppers grown in the glasshouse in pots of infested soil were not galled. Peanuts grown in infested field plots were generally resistant. The peanut roots were invaded by the nematodes, which caused considerable necrosis of the fine roots without any gall formation or development of the larvae

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in the roots. However some plants showed slight infection with galls typical of *M. hapla* Chitwood. The perineal patterns of adult female nematodes from these galls were of the *M. hapla* type, with characteristic punctation of the tail tip area (Taylor, Dropkin & Martin 1955).

It appears that the root knot nematode population of the Red Cliffs plots comprises *M. javanica* plus a small percentage of *M. hapla.*

II. materials and methods

(a) Treatments. — Four treatments were used in this trial — susceptible control crop, resistant crop, complete clean fallow, and summer fallow plus winter cover crop. The treatment layout was designed to take into account the past history of the plots.

(b) Material. — The variety Pan America was chosen for the control crop. This variety is susceptible to the root knot nematode but resistant to fusarium wilt, the only other soil borne disease frequently encountered in this area.

Hybrids of *Lycopersicon peruvianum* and *L. esculentum* were used as the resistant crop (Giles & Hutton, unpublished). Their resistance was not sufficiently uniform to prevent all reproduction of the nematodes, but they gave promise of commercial yields. Plants were regarded as resistant if they showed slight gall formation, or none, in the presence of large soil populations of nematodes. The resistance exhibited by any individual plant in the field depended on its genetic constitution and on the nature of the nematode population in its environment. The hybrid lines used in the first two seasons of the trial showed a rather high proportion of relatively susceptible segregants; subsequently, one of the parent lines, relatively fixed, was used to give more uniform field resistance. Hybrid lines also carried resistance to fusarium wilt.

Barley was used as the winter cover crop, though its susceptibility to nematode attack had not been determined at that time. The failure of earlier workers to distinguish between various species of root knot nematode led to some confusion over the susceptibility of various plant species to root knot.

(c) Plot layout and management. — There were eighty plots, each 15 ft by 9 ft, laid out in blocks of 16. Plots were separated by raised earth banks and spray irrigated to prevent irrigation water carrying

nematodes from one plot to another. They were hand weeded as often as necessary to control weed growth. Each treatment was replicated 4 times per block. Treatments with tomatoes had 9 plants per plot, spaced 5 ft by 3 ft. At transplanting, a mixture of one part of sulphate of ammonia to two parts of superphosphate was applied at the rate of 2 cwt/ac to these plots. Cover crop plots received superphosphate at the rate of 2 cwt/ac at seeding. At maturity the cover crop was chopped up and dug into the surface of the plots.

(d) Duration of the experiment. — Commencing in November 1951 with the planting of tomatoes, treatments were applied for three seasons. Then, in October 1954, one plot per block of each treatment was planted to susceptible tomatoes, and one to resistant tomatoes, to compare field results with estimates of treatment effects made during the course of the trial. At the same time the remaining plots were planted with a new series of treatments, which are not the concern of the present paper.

(e) Methods of assessing the effects of treatments on nematode infestation. — Treatment effects were estimated in various ways. Each April and November, 9 sites per plot, on the same 5 ft by 3 ft grid as the tomato plantings were made, were sampled to a depth of 9 in. with a soil auger. At the first sampling in April 1952 the samples were bulked to 1 per plot, but subsequently the whole 720 samples were kept separate on each occasion. Two-pound soil samples were used in tins to grow Pan America tomatoes.

Seeds were germinated in the soil samples taken in April. When the seedlings reached the first true leaf stage, all were carefully excavated, and one was replaced to be grown to maturity in a glasshouse. Root ratings for severity of gall formation were allotted to maturity on a scale from 0 (clean) to 5 (maximum infection). The remainder of the excavated seedlings were used for nematode counts. The nematodes in the roots were stained with acid fuchsin lactophenol (Goodey 1951) and then counted.

In November, Pan America seedlings from the same lot as used for the field plants were transplanted to the soil samples, one seedling per tin. It was not practicable to count nematode larvae in these roots since infection did not occur until the root systems were too large to be handled successfully. These plants were grown in a shade house and root ratings were made at maturity.

All tomatoes grown in the field were given root ratings. Thus the degree of resistance shown by the *L. peruvianum* hybrids in the field and the intensity of nematode infestation in the control plots could be assessed each season. In addition, yields were recorded of the resistant and susceptible tomatoes planted over all treatments in 1954-55.

III. RESULTS

(a) Nematode counts from plants in soil samples. — Statistical analyses of the results gave no indication that counts from barley and fallow samples differed, but differences between all other treatment combinations were highly significant. The logarithms of the counts were used as the variate to facilitate statistical analyses. The results are summarised in Table 1.

TABLE 1

Means (a) log. count and (b) count of nematodes in susceptible tomatoes (at first true leaf stage) grown in soil samples from treatment plots

Time of Sampling		Treatment Susceptible	Resistant	Barley	Fallow	Standard Error
April 1952	(a)	1.6944	1.1915	0.3510	0.1880	± 0.0947
	(b)	67	27	4	1	
April 1953	(a)	1.4672	1.0812	0.2569	0.1526	± 0.0555
	(b)	38	23	3	2	
April 1954	(a)	1.4119	0.9378	0.1289	0.1043	± 0.0539
	(b)	37	21	1	2	

No significance is attached to the apparent trend of a decrease in the count over the three seasons.

(b) Root ratings of plants in soil samples. — Analyses of these ratings gave results similar to those of the counts. In general, there was no difference between the barley and fallow ratings, which were significantly lower than those of the other treatments. Plants grown in soil from susceptible plots were most affected. November 1952 was the only occasion when barley and fallow ratings were found to differ significantly from each other, but it has not been possible to find any reason for this (Table 2).

TABLE 2

Mean root ratings at maturity of susceptible tomatoes grown in soil samples from treatment plots

0 — clean 5 — maximum infection

Time of Sampling	Treatment				Standard Error
	Susceptible	Resistant	Barley	Fallow	
April 1952	3.5	2.7	1.2	1.3	± 0.2
April 1953	2.7	2.0	0.6	0.4	± 0.1
April 1954	2.5	2.0	0.3	0.2	± 0.1
Nov. 1952	3.3	2.8	1.8	0.8	± 0.2
Nov. 1953	4.3	3.6	0.9	0.7	± 0.2

The relationship between ratings from susceptible and resistant plots remained fairly constant, with the resistant plots supporting a somewhat lower level of nematode population than the susceptible plots. Resistance of the hybrids was not complete; there was always sufficient susceptibility to permit multiplication of the nematodes. The ratings of barley and fallow plots showed a tendency to decrease over the period of the trial, but there was no indication that continuing the treatments for any definite period would entirely eliminate the root knot nematode. The effects of applying these treatments for another year are being investigated in an extension of this trial.

As the tables show, ranking of the four treatments with regard to both counts and ratings was the same. Examination of individual counts and ratings, however, showed that even within a particular treatment there was no relationship between any count and the corresponding rating. A high count was usually associated with a substantial rating, 2 or higher, but it was not possible to predict from the count what rating might be expected.

(c) Root ratings on field tomatoes. — Ratings allotted to field tomatoes during the trial are summarised in Table 3. In some cases it was not possible to allot ratings because of other root diseases or premature death of the plants; such cases are listed in a separate class. The ratings of susceptible plants show that the nematode infestation in the control plots built up quickly in the early stages of the trial. It is more difficult to interpret the ratings of resistant tomatoes. One factor which contributed to the variability of these figures was an increasing degree of resistance to root knot in the

hybrids used. The tomatoes planted in 1953 were much more resistant than those used earlier.

TABLE 3

Numbers of field tomatoes (susceptible or resistant) in various rating classes 1951-54

0 — clean 5 — maximum infection
M — no ratings possible

Season	Susceptible Rating Classes				Resistant Rating Classes			
	5	4-2	1-0	M	5	4-2	1-0	M
1951-2	113	13	29	25	25	10	109	36
1952-3	154	15	3	8	42	40	96	2
1953-4	152	16	0	12	5	16	155	4

The degree of root knot control effected by the application of the four treatments for three seasons is indicated by the ratings on susceptible and resistant field tomatoes grown in 1954-55 over all treatments (Table 4).

TABLE 4

Numbers of field tomatoes (susceptible or resistant) in various rating classes, season 1954-55

0 — clean 5 — maximum infection
M — no ratings possible

Susceptible Tomatoes 1954-55					Resistant Tomatoes 1954-55				
Original Treatment 1951-54	Rating Classes 5	4-2	1-0	M	Original Treatment 1951-54	Rating Classes 5	4-2	1-0	M
Susceptible	43	2			Susceptible		1	44	
Resistant	38	6		1	Resistant	8	5	30	2
Barley	8	15	22		Barley			44	1
Fallow	8	21	16		Fallow		1	44	

Growing of resistant tomatoes for three seasons, followed by a susceptible crop, gave a result not significantly different from continuous growing of susceptible tomatoes. Three years of complete fallow, or three years of partial fallow with barley cover crop, gave appreciable reduction of root knot in the subsequent susceptible crop. The proportion of resistant plants with rating greater than one was

significantly greater (P 0.01) on plots where resistant tomatoes had previously been grown for three years than on other treatment plots. The ratings of susceptible plants from susceptible plots suggested saturation of nematode infestation in control plots. Mean ratings of susceptible plants from the various treatment plots are shown in Table 5.

TABLE 5

Mean ratings of susceptible field tomatoes 1954-55

Previous Treatment: —	Susceptible	Resistant	Barley	Fallow
Mean Rating/Plant	4.9	4.8	2.0	2.4

Standard error ± 0.29
Least significant difference 1% = 1.3

(d) Yields of field tomatoes 1954-55. — The yields of resistant and susceptible field tomatoes in 1954-55 showed the same trends, though the yield of the resistant variety was always significantly higher than the yield of the susceptible variety on plots of the same original treatment. There were no significant differences between yields from barley plots and from fallow plots, nor between yields from susceptible plots and from resistant plots, but the differences in yield between the two groups fallow — barley and susceptible — resistance were highly significant (Table 6).

TABLE 6

Mean yields (lbs/plot) of field tomatoes 1954-55

Tomato Crop 1954-55	Original Treatment (S.E. ± 13.4) Susceptible	Resistant	Barley	Fallow	Mean
Susceptible	81.4	79.6	132.3	129.2	105.6
Resistant	129.5	121.6	160.3	153.2	141.1
Mean	105.5	100.6	146.3	141.2	

A very interesting feature of the yield figures is the lack of significant interaction between the treatments applied in 1954-55 and the original treatments applied to the plots.

IV. DISCUSSION

The various methods used to estimate the effects of the treatments

on nematode infestation gave similar results, but the differences between resistant and susceptible plots indicated by counts and ratings on plants in soil samples did not appear in the field results. Any reduction in nematode population which may have been caused by growing resistant tomatoes was not significant under field conditions.

The lack of interaction between treatments in the yield figures of Table 6 indicates that the effect of nematode infestation in reducing yield was not significant in this season. GILES & BROCK (1952) estimated yield losses due to nematode attack at about 80% for root rating 5, and about 6% for root rating 2. In these circumstances a significant interaction was expected. The authors believe that the lack of significance was due to above-average rainfall during spring and summer 1954-55, which apparently improved the yield of susceptible tomatoes on heavily infested plots by eliminating much of the stress imposed by a normal dry season on damaged root systems. Two points are quite clear from the yield figures; firstly, the resistant tomatoes were inherently higher yielding than Pan America, and secondly, fallowing, with or without cover crop, increased yields quite apart from any effect on nematode population.

Consideration of the root ratings of Table 4 suggests that all effects of the fallow treatments on nematode infestation would be nullified by growing one more susceptible crop on the plots, though there may by some carry-over to that crop of the fallow effect on yield.

As a means of estimating nematode infestation, counts of larvae in the roots by the staining technique appeared to have one major disadvantage. The number of nematodes present appeared to depend not only on the soil population of the nematodes, but also on the stage of invasion reached at the time of excavation of the plants. Where penetration of a root tip area was in its initial stages, some thirty or more larvae might be found at one site, with head embedded in the root tissue. Once penetration had been achieved, this population reduced to some two or three third stage larvae in a very short time. Thus the variability of counts in heavily infested samples was high, and, in general, lightly infested plots gave counts of zero to few, while a count of 10 to several hundred indicated a heavy infestation. The count was not a very sensitive measure of population. Counting was more difficult than root rating. Its advantage was that the time required for a result was short, and maintenance of plants in tins for extended periods was unnecessary.

Root rating gave a simpler and more effective estimate of nematode

infestation. The lack of relation between individual counts and ratings was explained by the factors outlined above. Ratings were subject to seasonal variations, but could be used at any time of the year if facilities for growing plants were available. Ratings from plants grown in winter were consistently lower than from summer grown plants; even the latter did not show the same degree of galling as field plants grown at the same time. Ratings from the samples tended to over-emphasise the effects of barley and fallow treatments on nematode infestation, as compared with field results. A low initial infestation appeared more important in the tins than in the field.

All the ratings were made at maturity so that ratings from tins might be compared with field ratings. Since swellings are produced on the roots soon after penetration of the nematodes, ratings are possible at a much earlier stage, eliminating a great deal of work. It is possible that earlier ratings would follow field results more closely, since host-parasite relationships should be less affected by conditions peculiar to growth in tins if the plants were removed earlier.

No indication of gall formation on barley roots was discovered in the field plots, despite numerous examinations. Barley grown in infested soil in the glasshouse did not develop galls. Sasser (1954) found that *M. javanica* attacked barley, but gave lower root ratings than some other species on this particular crop. In view of the reduction in nematode activity found to occur during winter, and the reduced ratings shown by tomatoes under glasshouse conditions, it is not certain that barley is completely resistant to the root knot at Red Cliffs, though such appears to be the case. In any event it is unlikely that barley would ever increase nematode infestation under field conditions there.

The existence of races with different host ranges within species of *Meloidogyne* has been discussed by Allen (1952) and by Martin (1954). Repeated growing of resistant plants in the Red Cliffs plots appeared to induce a change in the nematode population, increasing its ability to break the resistance. There was no indication that this change was due to an increase in the population of *M. hapla*; all nematodes examined from infected resistant tomatoes appeared to be *M. javanica*. Thus it appears probable that selection of a race of *M. javanica* has occurred in resistant plots during this trial. More work on this effect is necessary as other explanations are possible. A combination of pathogens may be involved. Young (1939) suggested that root knot increased the severity of fusarium wilt on tomato, but

McClellan & Christie (1949) could find no such relationship; however associations between nematodes and fungi have been demonstrated in the case of fusarium wilt of cotton (Holdeman & Graham 1953) and black shank of tobacco (Sasser, Powers & Lucas 1953). Some resistant tomatoes have been affected by root rots of unknown origin in the field plots at Red Cliffs.

Little is known of the mechanism of resistance in *L. peruvianum* and its hybrids. The roots of the hybrids are apparently heavily invaded by nematode larvae since no larvae are recovered from heavily infested soil after the hybrids have grown in the soil for a few weeks, but there is no necrosis of the roots as is shown by infested peanuts.

V. conclusions

Resistant tomatoes always produced a reasonable crop, but they did not reduce nematode population to a level which would make the land suitable for susceptible crops. Repeated growing of resistant tomatoes on the same land was undesirable; crop rotation with another non-susceptible crop appeared to be necessary for continued production. The decreased resistance with repeated planting did not appear to be due to a mixed population of two species of root knot nematodes.

Fallowing significantly reduced nematode infestation when applied for a number of years. Tomatoes grown in 1954-55 gave significantly higher yields on land previously fallowed than on other plots. These increased yields could not be attributed to the reduced nematode infestation brought about by fallowing. When the fallow was broken by susceptible tomatoes the nematode population increased rapidly, and the susceptible crop would probably succeed only in the first season. Fallowing is not a practicable treatment in irrigation areas with high land values, but may be useful in other areas. The difficulty of maintaining a clean fallow, and the susceptibility of many weed species to root knot, reduce its usefulness.

Growing a green manure winter crop in conjunction with summer clean fallow gave the same result as complete clean fallow.

VI. acknowledgments

The authors are indebted to Dr. J. W. Seinhorst, of the Institute of Phytopathological Research, Wageningen, Holland, for identification of the principal root knot species as *M. javanica*. The results of the trial were statistically analysed by Mr. R. Birtwistle, of

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SUMMARY

The effects of four systems of field management on *Meloidogyne javanica* (Treub) Chitwood infestation of tomatoes were studied over three seasons 1951-54. Counts of nematode larvae in tomato roots, root gall ratings of tomato plants and yields of tomatoes, were used to estimate these effects.

Growing of susceptible tomatoes in the same soil year after year gave a uniform severe infestation which usually depressed yields.

Growing of nematode resistant hybrid tomatoes assured good yields but did not reduce the nematode population to make the land suitable for susceptible crops, since the resistance varied from plant to plant, permitting some reproduction of the nematodes. Resistance was decreased by repeated planting.

Clean fallow for three years reduced nematode infestation significantly. Fallowing had the effect of increasing yield quite apart from any increase due to reduced nematode attack. When the fallow was broken by a susceptible crop the nematode population increased rapidly.

Summer fallow with winter barley cover crop gave the same result as complete clean fallow.

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HOPLOLAIMUS PROPORICUS N.SP. (HOPLOLAIMINAE: TYLENCHIDA) *)

BY

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Early in 1951 the Cameroons Development Corporation sent for identification, some nematodes extracted from the rotten roots of seedlings of the oil palm, *Elaeis guineensis* Jacq. suffering from the disease known as 'blast'. Later whole root systems were sent and then in 1952 further root material of both *E. guineensis* and *E. melanococca* Gaertn. came from the West African Institute for Oil Palm Research, Nigeria. Roots were extensively rotten and from them a few specimens of a species of *Hoplolaimus,* which appeared to be new to science, were obtained. The eelworms were found in the short laterals and not in the advancing edge of the rot and it appeared unlikely that the eelworms were responsible for the disease. This view is supported by BULL (1954) who found the same species of *Hoplolaimus* associated with roots of both diseased and healthy seedlings of oil palm in Nigeria again only in small numbers.

The eelworms look remarkably like short curved pieces of fibre or glass rod when seen in fixative under low magnification. They have an opacity which separates them immediately from other nematodes. This point has some importance because at first glance their nature is not obvious and they are therefore liable to be overlooked.

There are several interesting features shown by this new species, but two are outstanding. Firstly there is no lateral field, for except at the extremities of the eelworms, the annules run without interruption right round the body. Secondly the excretory pore is found very far forward at about the level of the corpus. This second feature is used in making the specific epithet of the nematode which is hereby named *Hoplolaimus proporicus* n.sp.

Dimensions. — 16 ♀♀ L = 1.05-1.55 mm. a = 25-33: b = 5.7-9.7: c = 49-164: V = 55-59. 4 ♂♂ L = 0.96-1.12 mm. a = 29-32: b = 6.2-7.1: c = 32-36.

Holotype ♀ L = 1.55 mm. a = 32: b = 9.7: c = 112: V = 57.

Allotype ♂ L = 1.08 mm. a = 30: b = 7.1: c = 35.

The head, usually with four annules, is offset from the body by a

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slight constriction. There are six lips of which the lateral ones have slit-like amphidial openings towards their outer edges and are smaller than the others. No papillae were discernable on the lips, which are well rounded with distinct grooves between them. There is some longitudinal division of the lowest of the annules but there is nothing like the extensive 'tiling' which was figured by Cobb (1923) in his drawings of the head of *H. coronatus*. The skeletal anatomy of the head is complex and can be best understood by reference to figure 1 B-F. The so-called basal plate is divided, primarily, into six arms from which two sets of ribs arise; centrally those which are fused around the hole through which the spear is guided, and peripherally those supporting the junctions of the lips. These latter curve inwards and fuse with the tops of the central ribs. The dorsal and ventral arms of the basal plate are each further sub-divided into three. At a slightly lower level, just at the basal constriction of the head, the plate extends right to the edge of the body so that the whole head and front part of the body must form a strong tough structure. There is a peripheral, collar-like, posterior projection of the head skeleton; the muscles from the base of the spear are attached here and at the outer edges of the plate. The spear is powerful and has massive knobs which, in shape, are strongly reminiscent of a tulip bulb. The fore and aft parts of the spear are approximately equal in length and the whole is about 41 μ long.

The annulations of the body are well marked, the striae being about 2 μ apart on the cuticle and twice as frequent on the sub-cuticle. The annulations go right round the body along most of its length. On the front end, on the tail and just anterior to the anus the striae are interrupted in the lateral line but there is no true lateral field (Figure 2 L-N.) The phasmids (Fig. 2 N, O) are large sub-spherical sacs similar to those of *H. coronatus* and, they also, are situated in the erratic manner of that species. Of fifteen females examined, seven had the left phasmid placed anteriorly and the right phasmid posteriorly while the other eight had the positions reversed. The position of the anterior phasmid varied from 24% to 52% of the body length with a mean of 32% and the posterior one varied from 58% to 89% with a mean of 79%. To some extent the phasmids appear to be equidistant, anteriorly and posteriorly, of the vulva. In the four males all the right phasmids were anterior, varying in position from 33% to 48% with a mean of 38% and the left phasmids posterior, 75% to 83% with a mean of 79% of the body length.

The tail is relatively short in the female, occasionally very short

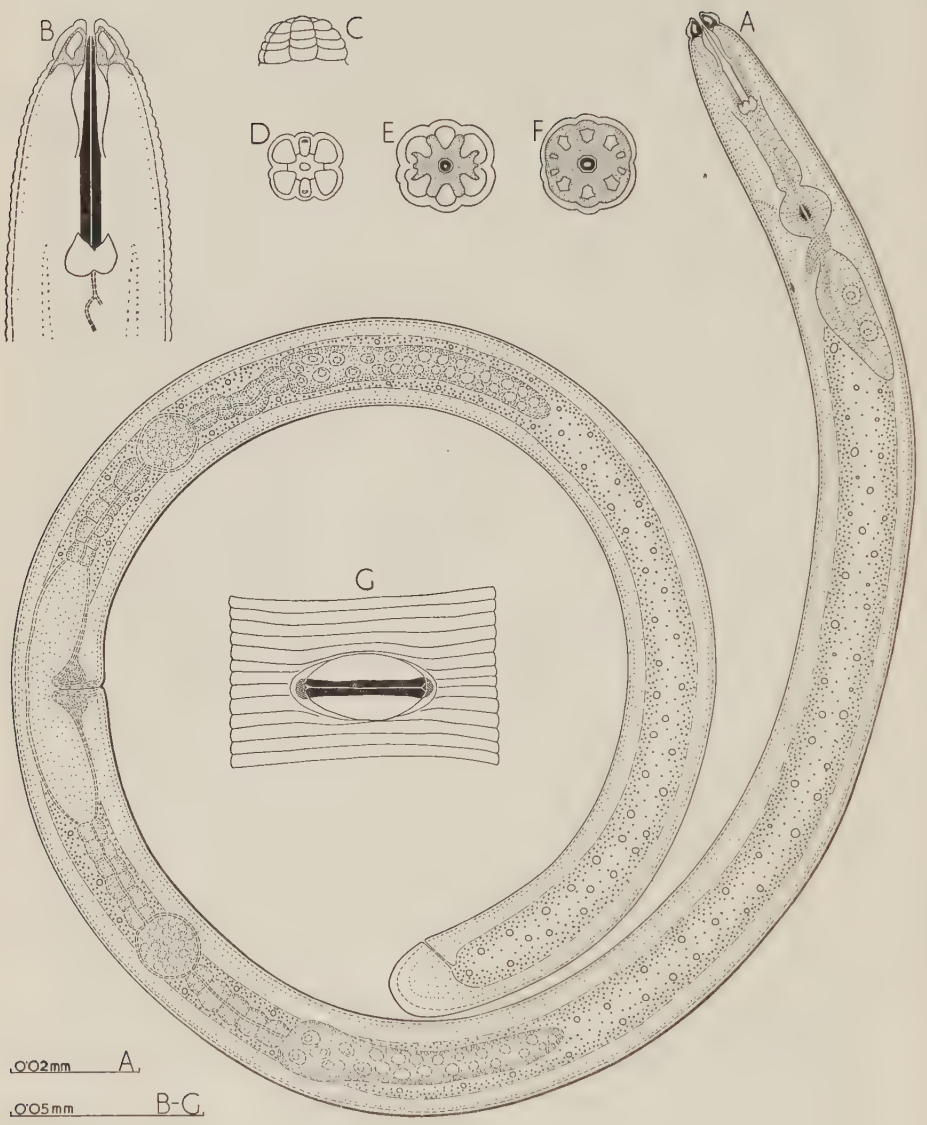

Fig. 1. *Hoplolaimus proporicus*. A: female eelworm, note anterior position of excretory pore. B: head end showing spear and skeleton of head. C: head in lateral surface view by incident illumination. D: end-on view of head. E & F: basal plate of head skeleton at two different levels. G: vulva in ventral view by incident illumination.

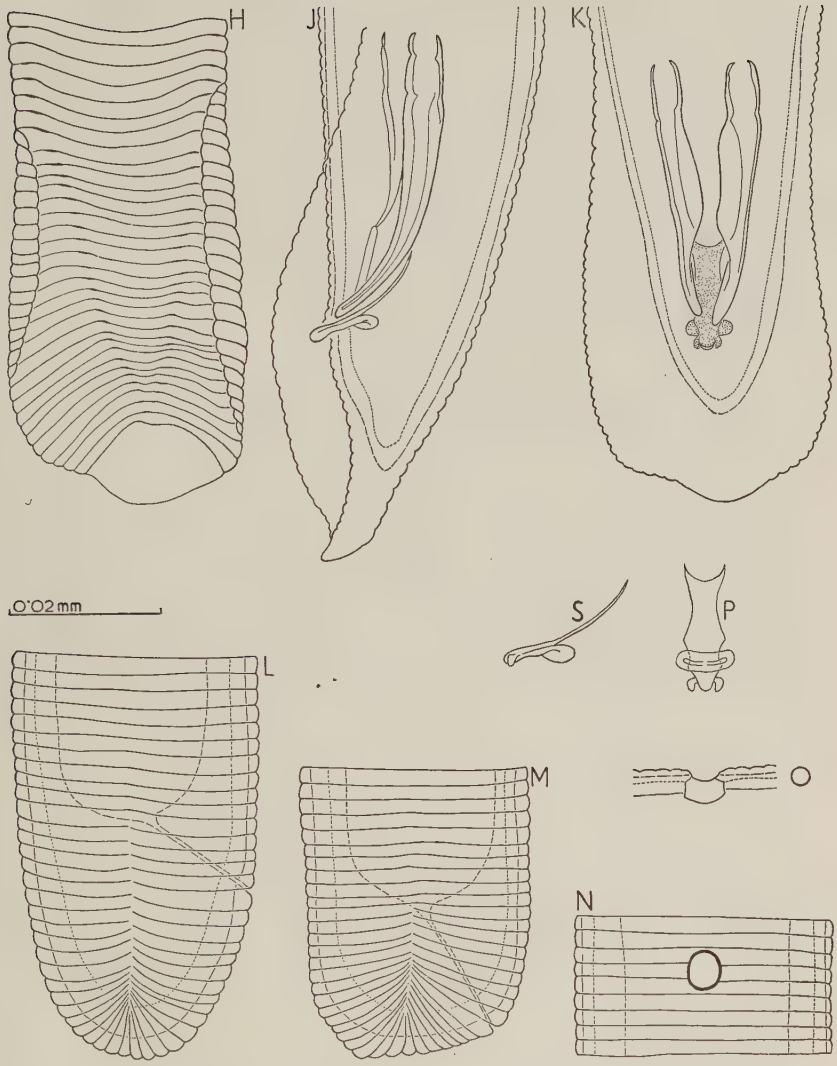

Fig. 2. *Hoplolaimus proporicus*. H: male tail in ventral surface view showing the enveloping bursa. J: male tail in lateral view to show relation of spicules, gubernaculum and telamon. K: male tail in ventral view showing relation of spicules to gubernaculum. M & N: female tails in lateral view showing variation in length and the absence of lateral fields. N & O: large phasmid in surface view and in section. P & S: gubernaculum in dorsal and lateral view.

indeed (Figure 2 L, M). The excretory pore opens at about the level of the corpus. The hemizonid is not immediately anterior to the excretory pore as it is in most other Tylenchida but is situated at the point one would expect to find both it and the excretory pore, that is, about opposite the middle of the post-corpus. This wide separation — there are about twenty annules intervening — of excretory pore and hemizonid suggests that it is unlikely that there is any functional connection between the two, though it sheds no further light on the possible function of the hemizonid.

The structure of the oesophagus and intestine call for little comment, they are depicted in Figure 1A.

The female reproductive system is paired, equal and outstretched. The vulva, about a half-body width in length, opens transversely on the ventral surface just behind the middle of the body. It connects to the middle of the uterus by a short vagina. Each horn of the uterus is joined to an ovary by a thick-walled oviduct which has a more or less spherical spermatheca half way along its length. No eggs have been seen in any of the eelworms but the spermathecae were full of sperms. Most of the ovaries were comparatively undeveloped; they are illustrated so in Figure 1A and in the measurement data the extents of the gonads have been omitted. The details of the male gonads were rather difficult to make out and could only be seen properly in two of the four specimens. The bursa of the male tail begins at the level of the spicule heads and extends round the tip of the tail. The spicules are curved ventrally, have inwardly curving flanges, and their structure is shown in Figure 2 J, K. The gubernaculum is depicted in Figure 2 J, K, S, P. On either side of its protrusible tip is a projection, the titilla of THORNE (1949). The males of *Hoplolaimus* have a telamon, a thickening of the ventral wall of the cloaca, which appears to lie between the spicules and is usually not easy to see. In *H. proporicus* there is a telamon and the relationship between it and the spicules is shown in Figure 2 J.

Type host. Roots of *Elaeis guineensis* Jacq., the oil palm.

Type locality. British Cameroons.

Type specimens. Holotype ♀ No. 55/2/11, Allotype ♂ No. 55/2/10, in collection of Nematology Department, Rothamsted Experimental Station.

Discussion. — The genus *Hoplolaimus* contains four species. The type species, *H. tylenchiformis* Daday, 1905 was imperfectly described and recently ANDRASSY (1954) has redescribed it from the type specimen.

The other species are *H. coronatus* Cobb, 1923, *H. uniformis* Thorne, 1949 and *H. proporicus*. According to Thorne one of the characters peculiar to *Hoplolaimus* is that the annules of the head are divided longitudinally so that the head appears to be covered by minute blocks. This is true for *H. uniformis* but although Cobb depicted the head of *H. coronatus* in a somewhat similar manner, specimens of *H. coronatus* which the author has examined by surface illumination, have only the lowest and sometimes the next annule subdivided longitudinally. Cobb said that the lowest annule was subdivided into 32 parts. According to Andrassy *H. tylenchiformis* has only the lowest annule subdivided and that into about 24 parts. In *H. proporicus* there is almost no subdivision at all, even of the lowest annule.

H. proporicus differs from the other species in the genus in having no lateral field; the annules go right round the body except at each end of the eelworm where they are slightly interrupted in the lateral line.

Within the genus, *H. proporicus* and *H. coronatus* are alike in the shape of the head, form of the spear, and type and erratic position of the phasmids. *H. tylenchiformis* is similar except that the phasmids are, according to Andrassy, not so strikingly developed and are apparently placed on the tail. *H. uniformis* is different in the shape of the head, form of spear, subdivision of the head annules and position and size of the phasmids.

ZUSAMMENFASSUNG

Hoplolaimus proporicus n.sp. wird beschrieben und abgebildet. Der Nematode wurde im Zusammenleben mit den Wurzeln der Ölpalme *Elaeis guineensis* gefunden. *H. proporicus* weicht von allen *Hoplolaimus*- Arten insofern ab, als er keine lateralen Felder aufweist und die Ringe rund um den Körper verlaufen mit Ausnahme an den beiden Enden des Nematoden, wo sie in der Seitenlinie etwas unterbrochen sind.

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STUDIES ON ROOT KNOT NEMATODES OF THE GENUS *MELOIDOGYNE* IN THE GOLD COAST

PART II

THE EFFECT OF SOIL MOISTURE CONTENT ON SURVIVAL OF THE ORGANISM [1])

BY

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GODFREY (1926) in greenhouse experiments on tomato plants, found a marked decrease in severity of root-knot attack at moisture contents below 40% of saturation and above 80% saturation. Unfortunately root-knot counts were still high at these limits (30% saturation was the lower limit) and plant growth suffered, and he concluded that soil moisture played only a small part in root-knot development, so long as moisture content was favourable to the growth of the crop. Later experiments (GODFREY, OLIVEIRA and GITTEL 1933) in which the survival of root-knot nematodes was studied in soils of varying moisture contents, in the absence of a host plant, showed that drying of soil and alternate wetting and drying materially hastened death of the root-knot organism in egg and larval stages. Excess moisture hastened exhaustion of vitality of larvae, and where jars were sealed with high moisture content permitting of continuous decomposition of organic matter, death of nematodes was accelerated. In these experiments soils in tubs and jars were allowed to dry out over a period of 20 weeks; at the end of this period the two soils used still retained, respectively, 40% and 28% relative wetness. The moisture equivalent of the soils used was more or less the same as the field capacity; relative wetness was the ratio of the moisture content to the moisture equivalent, expressed as a percentage.

PARRIS (1948) showed that galling by root-knot eelworm was more

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severe on potato tubers, and less severe on potato roots, in wet soil than it was in dry soil, but pointed out that in wet soil new root formation would be quicker and could more than keep pace with the damage done by the eelworm. In these experiments, too, 'dryness' was limited by plant growth requirements and was never less than 24-27% (moisture equivalent approximately 32%).

Crittenden (1953) in experiments in Delaware, in which land was cultivated once every 2 weeks from May 1st to the time of planting (May 15th, June 1st, June 15th, July 1st) showed that cultivation during June reduced nematode populations on soya, but no soil moisture content figures were given.

In a series of greenhouse tests at Achimota (University College of the Gold Coast) under conditions of varying soil moisture independent of plant growth requirements, the survival of the root-knot organism in the form of egg-masses, larvae, and naturally infested material containing all stages of the parasite has been studied in certain light sandy soils. The conclusions drawn from these experiments have been tested under field conditions in a market garden at Accra, Gold Coast.

LABORATORY WORK

In the light sandy soils used in these experiments, moisture equivalent (the moisture retained by a soil after centrifuging at 1000 × 'g' for 10 minutes) was far below field capacity, and the standard taken for saturation was therefore the moisture holding capacity.

Air-dried sterile soil, conditioned to approximately the desired moisture content by the addition of calculated amounts of water, was inoculated with the organism to be studied, and the actual moisture content obtained by determining the loss in weight of a sample on oven-drying at 105° C. for 18 hours. The inoculated soil was then held in sealed Kilner jars, turned each day, for the experimental period (12 hrs. up to 10 days) after which time it was transferred to 3½" pots in a netted "greenhouse", watered if necessary and planted with young tomato plants. Inoculation with root-knot eelworm larvae was by pipetting a suspension of newly-hatched larvae in distilled water, the inoculum containing 500-600 larvae per jar, and was done before determining the soil moisture content. Inoculation with egg-masses was done after determining the soil moisture content, by transferring egg-masses to the soil on the point of a needle, 3-4 egg-masses per jar. The soil used had a moisture holding capacity of

32% of its oven-dry weight, and a moisture equivalent of 19.0%. Soil temperatures varied from 25-28° C.

Naturally-infested soil from root-knot infested areas in the field, was conditioned either by the addition of water or by air-drying on the laboratory bench for ½ to 3 hours and turning at intervals. Actual moisture contents were determined as before, and the soils were held in sealed Kilner jars, afterwards being transferred to 3½" pots in the "greenhouse". Moisture holding capacity of these soils varied from 27% (Expt m/3) to 25% (Expts m/4, m/5), with moisture equivalents of 16% and 14.5% respectively. Soil temperatures ranged from 25-30° C.

Treatments were assessed by counting the knots formed on roots of the indicator plants. These observations were carried out within 30 days of planting, in order that primary infections only should be counted.

Soils were held at known moisture contents for periods determined by preliminary experiments not reported here. Within the range of holding times examined, however, the effect of time on treatments was small compared with the effect due to varying the soil moisture, and the data were combined to give greater replication. Similarly, in experiment m/2 where the effect of sealing or not sealing the jars was examined, differences were small and the data were again combined.

TABLE I

Effect of low soil moisture on survival of Meloidogyne larvae and egg-masses

Experiment	Soil	Moisture percent. of saturation	Mean number of knots on indicator plant roots	
			Soil inoculated with larvae; held 12-48 hours, 9 replicates	Soil inoculated with egg-masses; held 1-5 days, 9 replicates
m/1	Sterile loam	6.4	0.0	0.0
		9.6	0.1	0.1
		13.0	0.9	79.8
		16.5	49.0	78.2

Tables I and II show that larvae were unable to survive in a soil held at less than 4.2% moisture (13% of saturation) for 12-48 hours. Numbers were reduced in soil held at 100% saturation (moisture holding capacity) for 10 days but were not affected by 10 days in soil at 20% moisture (62% of saturation). Egg-masses failed to survive

1-5 days in a soil with less than 3.1% moisture (10% of saturation). Few survived 5-10 days at 100% saturation (moisture holding capacity) but were not affected by 10 days at 20% moisture (62% of saturation).

TABLE II

Effect of high soil moisture on survival of Meloidogyne larvae and egg-masses

Experiment	Soil	Moisture percent of saturation	Mean number of knots on indicator plant roots	
			Soil inoculated with larvae; held 10 days, 4 replicates	Soil inoculated with egg masses; held 5-10 days, 8 replicates
m/2	Sterile loam	21.8	30.8	53.8
		62.0	35.5	57.9
		100.0	22.5	2.1

A complete control of natural infestations was obtained in soil held for 2-5 days at 2.9% moisture (11% of saturation), and an appreciable reduction occurred at 3.7% moisture (14% of saturation) and at 100% saturation (Table III). Where the organism was protected by unrotted plant material, there was no control after 2-5 days at 3.2 to 25% moisture (13-100% of saturation), but at 1.3% moisture (5% of saturation) control was virtually complete (Table IV).

In this last experiment infection of the indicator plant roots was so heavy that results were assessed by grading instead of counting the knots.

TABLE III

Effect of varying soil moisture content on a naturally occurring Meloidogyne population

Experiment	Soil	Moisture percent. of saturation	Mean number of knots on indicator plant roots
			Natural infestation of soil; held 2-5 days. 10 replicates
m/3	Root-knot infested light sandy-soil	10.7	0.0
		13.6	17.4
		18.3	70.6
		29.0	75.6
		70.5	47.6
		100.0	22.9

TABLE IV

Effect of varying soil moisture content on a Meloidogyne population protected by plant material.

Experiment	Soil	Moisure percent. of saturation	Mean root-knot grading of indicator plant roots: Infested soil plus root-knot material; held 2-5 days, 5 replicates
m/5	Root knot infested light sandy soil to which 1/4″ cubes of root-knot material were added.	5.1	1
		12.9	10
		17.2	10
		35.6	9
		76.5	9
		100.0	9

FIELD WORK

Field work aimed at the reduction of soil moisture in the top 6-8″, below the level determined in the laboratory as the minimum for eelworm survival. The treatment consisted of forking over the soil at intervals prior to planting tomatoes as an indicator crop. Control plots were left undisturbed, but were watered sparingly from time to time to prevent drying-out of the surface soil. The moisture content of the soil was determined at 2″ levels down to 14″ using a 1½″ diameter core soil sampler, the 2″ sections being cut off into polyethylene bags and weighed immediately. Loss in weight on oven-drying at 105° C for 18 hours then gave the moisture content of the soil as taken and this was converted to 'percent of saturation'. Effectiveness of the treatment against root-knot was assessed by grading the degree of knotting on the roots of the indicator plants after a growing period which varied in different experiments from 7 to 11 weeks. Soil temperatures were for the most part in the range 26-31° C, but rose on occasion up to 35° C.

Distribution of the root-knot organism at different levels was studied, using a 4″ diameter tube which could be hammered into the ground to a depth of 18″. By means of a device using a hydraulic jack to push out the soil-core from the tube, samples were cut off into 2″ layers (compaction in this soil being negligible) which were then potted-up with small tomato plants. The number of knots formed on the roots of these plants gave an indication of the relative infestation of the soil at different depths. Results are presented in Tables V to VII.

TABLE V. *Expts. C/1, C/2, C/3 summarised*

Effect on survival of field infestations of root-knot eelworm, of drying cultivations during August '55 to January '56

Accra, Gold Coast

Treatment	Mean root-knot grading (scale 1-10) on tomatoes planted at intervals after treatment commenced				
	1 Month (Aug./Sept.)	2 Months (Aug./Oct.)	3 Months (Aug./Nov.)	1 Month (Nov.)	1 Month (Jan.)
	Expt. C/1			Expt. C/2	Expt. C/3
Fallow plots, forked over twice a week before planting	5.06	4.41	1.27	2.24 ± 0.15	3.72 ± 0.22
Fallow plots, not disturbed but occasionally watered to prevent drying	6.50	7.04	2.00	5.45 ± 0.31	6.07 ± 0.27
Statistical criteria					
General mean	—	—	—	3.74 ± 0.19	4.91 ± 0.30
Standard error of mean difference	—	—	—	± 0.64	± 0.77
Significant at	—	—	—	< 0.001%	< 0.01%
Meteorological data, monthly means					
Rainfall in ins. and (in brackets) number of wet days	August 0.59 (5) September 1.73 (11)	October 7.93 (5) November 0.75 (4)		November 0.75 (4)	January 0.11 (1)
Air temperature °C mean maximum	August 27°C September 28°C	October 29°C November 30°C		November 31°C	January 31°C
Relative humidity 12.00 noon	August 77 September 75	October 73 November 66		November 66	January 64

TABLE VI

Moisture content of soil in Experiments 55 C/1, 2, 3

Treatment	Depth of Sampling inches	Moisture content (% of saturation)				
			Expt. 55 C/1		Expt. 55 C/2	Expt. 55 C/3
		Sampled 24.8.55	Sampled 24.9.55	Sampled 29.10.55	Sampled 30.11.55	Sampled 27.1.56
Plots forked over twice a week	0-2	Initial sampling before treatment commenced	3	1	2	1
	2-4		11	3	2	2
	4-6		29	22	8	6
	6-8		33	32	13	10
	8-10		32	33	18	14
	10-12		36	35	20	19
	12-14		38	36	27	21
Plots not disturbed but watered to prevent drying-out	0-2	4	33	20	15	12
	2-4	19	35	23	16	17
	4-6	29	37	29	20	17
	6-8	33	39	31	25	18
	8-10	32	35	33	27	23
	10-12	35	36	35	31	22
	12-14	37	41	42	28	26

TABLE VII

Vertical distribution of root-knot eelworm in soil 10 weeks after tomatoes lifted. 4″ cores. Accra 1955

Depth of Sampling	Observations on tomatoes grown in soil samples			% of Infection
	Number of knots per plant Rep. A.	Rep. B.	Mean Number of Knots	
0-2″	0	0	0.0	0.0
2-4″	18	11	14.5	19.2
4-6″	25	39	32.0	42.4
6-8″	17	24	20.5	27.2
8-10″	8	7	7.5	9.9
10-12″	1	1	1.0	1.3
12-18″	0	0	0.0	0.0

An experiment on low-lying, poorly drained land during a season of intermittent rain (Expt. C/1) showed a slight reduction in the severity of root-knot on tomatoes grown on plots which had been forked over twice a week for 1, 2 and 3 months. Soil sampling at different depths on a dry day showed that only the top 4″ had dried out below the critical level for eelworm survival.

When plots on a well-drained slope (Expt. C/2) were forked over twice a week for one month during a dry season with no rain, a highly significant and spectacular reduction in root-knot severity on

the subsequent tomato crop was demonstrated. Soil sampling at different depths at planting time showed that drying had been effective to a depth of 8″. (In this soil, sampling at 2″ depths to 18″, 10 weeks after a tomato crop was lifted, showed that 88.8 % of the root-knot infestation occurred in the top 8″ — see Table VII).

A similar trial only a few yards away was deliberately heavily infested by digging-in the galled roots of a previous tomato crop. Forking over alternate plots twice a week for one month before planting, again gave a marked reduction in root-knot on the subsequent tomato crop even under these severe conditions, although the level of infestation was higher on both treated and control plots.

In Expt. C/1, although material differences in severity of root-knot were apparent between the 'dry' and the control plots, the degree of galling was still high after 1 and 2 months treatment. (After 3 months' treatment the issue was complicated by a general reduction of root-knot severity). Table 6 shows that, during this 3-month period, drying of the soil on the dug plots was effective (i.e. 13 % of saturation or less) only to a depth of 4″. In experiments C/2 and C/3 where a valuable degree of control was achieved, on the dug plot moisture reduction was effective to depths of 8″ and 10″ respectively (Table VI).

These experiments show that reduction of the soil moisture below the critical level for eelworm survival can be accomplished simply under practical conditions, and can give a worthwhile measure of control. To be effective, this treatment should be undertaken during a dry season such as that obtaining in Accra from November to February. Further work is necessary to apply these findings to other soils and localities, and to determine the optimum number, and length of time, of cultivations.

ACKNOWLEDGEMENTS

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SUMMARY

In laboratory and greenhouse experiments to determine the limits of tolerance of the eelworm to soil moisture, root-knot eelworm larvae and egg-masses were shown to be highly susceptible to death by desiccation. Egg-masses were also

somewhat intolerant of excessive moisture. Where nematodes were protected by plant material both these extremes had less effect. The critical moisture content appeared to be 3-4% (10 to 13% of saturation) below which larvae and eggs were unable to survive, except where protected by plant material.

In field experiments it was shown that simple cultivations during the dry season November-February were sufficient to reduce soil moisture to or below this critical level, and that a practical measure of control (as determined by assessing the severity of attack on the following crop) did, in fact ensue.

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(*Nematologica* II (1957) : 123-125)

THE HATCHING RESPONSE OF *HETERODERA MAJOR* (O. SCHMIDT) TO CERTAIN ROOT DIFFUSATES *)

BY

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It is well known that the larvae of most species of *Heterodera* can be stimulated to hatch and emerge from the cysts which contain them when the latter are placed in leachings from the roots of some of their host plants. These leachings are usually referred to as "root diffusates". Since the work of TRIFFITT (1930) many workers have attempted to assess the effect of various root diffusates on the eggs of several species of *Heterodera*. There are, however, some species which show no hatching response to treatment with host plant root diffusate, namely, *Heterodera major* O. Schmidt and *Heterodera göttingiana* Liebscher. Recent work (WINSLOW 1955, HESLING 1956) has shown that if the eggs in the cysts of *H. major* are dried, their hatchability in water is reduced. A similar effect of drying is found with *H. schachtii* (WALLACE 1955) and with *H. glycines* (SPEARS 1956) and is probably also to be found with other species of Heterodera.

Cysts of *Heterodera* are often obtained by flotation from dried soil. As it was possible that cysts of *H. major* which had been dried were used as test material by some workers searching for an active root-diffusate, it was decided to test out root diffusates from various host plants on wet cysts of *H. major*. Cysts of this species which had never been allowed to dry were obtained by a soil washing and elutriation technique (HESLING 1956) and batches of twenty five large cysts were kept in water in a watch glass at 2° C. At this temperature the hatch was very low.

Root diffusate was collected from actively growing plants of the species listed in the table I. The method of collection of the root diffusate followed that described by FENWICK (1949) and approximately one hundred ml. of root diffusate were collected at each leaching. Distilled water was used to leach the pots, and the leachate was stored in clean, corked bottles at 2° C.

*) Received for publication: October 26, 1956.

TABLE I

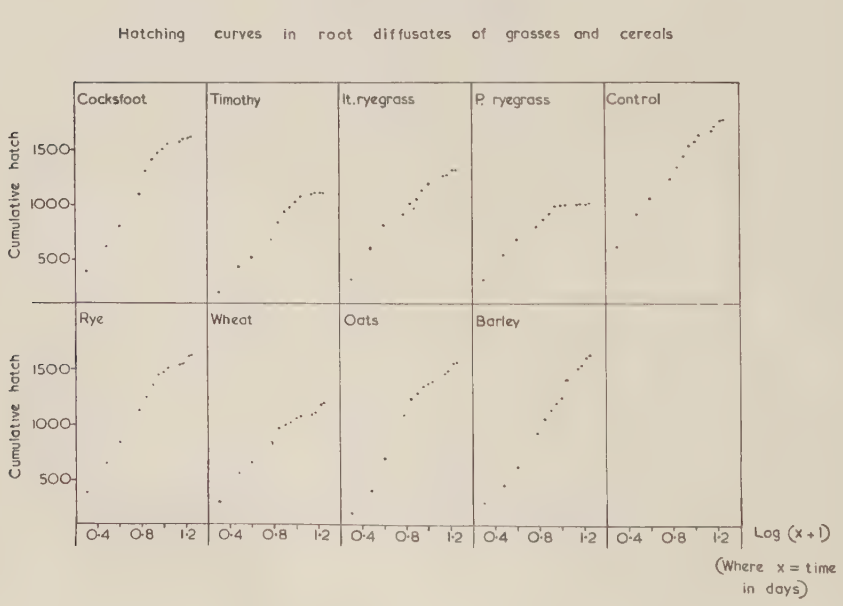
Plants from which root diffusates were obtained

Host		*Variety*
Oat	*Avena sativa*	S147
Wheat	*Triticum vulgare*	Bersee
Barley	*Hordeum sativum*	Proctor
Rye	*Secale cereale*	Petkus Spring
Perennial Ryegrass	*Lolium perenne*	S23
Italian Ryegrass	*Lolium italicum*	S22
Cocksfoot	*Dactylis glomerata*	S143
Timothy	*Phleum pratense*	S48
	Control pot of fallow soil	

Duplicate batches of the stored cysts of *H. major* were treated with root diffusate in early July and then kept at 21.5° C, hatched larvae being removed daily with a pipette. Fresh root diffusate which had been allowed to reach room temperature was added to each batch after the removal of the larvae.

For each root diffusate the mean cumulative hatch from the duplicate batches of cysts was plotted against the logarithm of the time in days, (Figure 1).

Fig. 1.



In each the hatch falls off after eight to ten days, and there is little difference between the rates of hatch (shown by the slope of the curve) or in the total number of larvae obtained. It appears that none of the root diffusates tested possesses stimulating properties. The hatching stimulus seems to be due to the influence of temperature since statistical analysis shows that there are no significant differences between the cumulative larval counts for each root diffusate treatment. The addition of freshly collected root diffusate when hatching had almost ceased did not provide any further hatching stimulus.

DISCUSSION

Although the above experiment indicates that the hatching of *H. major* is not due to the stimulus of root diffusate, it is possible that a hatching stimulant is produced from the plant roots but is ephemeral, being destroyed by storage or acting only in a region close to the plant root. The total hatch in an experiment similar to the above is usually less than 15% of the total cyst content. In the field, in fallow soil, the hatching period of *H. major* extends from March to July and over 60% of the cyst contents disappear during this time (unpublished work). Goffart (1956) has observed that the larvae hatch from some cysts and not from others. There may be differential maturation between and within cysts and this may provide extended hatch of eggs in the spring and early summer, but the reduction of hatching in the autumn and winter seems to indicate that this eelworm has a dormant period at this time.

ZUSAMMENFASSUNG

Um die Ursachen für das Schlüpfen von Larven des Hafernematoden kennen zu lernen, wurden Wurzeldiffusate von acht Getreide- und Grasarten im Vergleich mit Bodenwasser als Kontrolle geprüft. Beachtliche Unterschiede in den Schlüpfzahlen traten bei den einzelnen Versuchsreihen nicht auf. Wahrscheinlich wird das Schlüpfen durch eine Temperaturerhöhung gefördert.

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ÜBER DIE AKTIVIERENDE WIRKUNG VAN AMINOACRIDINEN AUF *HETERODERA SCHACHTII* *)

VON

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Die grundlegende Frage nach dem physiologischen Mechanismus der Reizperzeption und Reizbeantwortung bei der Zystenaktivierung von *Heterodera schachtii* durch chemische Reize ist bisher ungeklärt. Zahlreiche Schlüpfversuche zeigen, dass die enzystierten Larven dieser Nematodenart offenbar durch sehr verschiedene Reizqualitäten zum Verlassen ihrer Zysten angeregt werden (RADEMACHER, 1930). Ein allgemeines Prinzip für die Zusammenhänge zwischen chemischer Eigenart einer Substanz und ihrer spezifischen Reizwirkung lässt sich nicht erkennen.

Im Rahmen einer grösseren Versuchsreihe, über deren Ergebnisse in einer späteren Veröffentlichung berichtet werden soll, führten wir einige Schlüpfversuche mit Aminoacridinen durch, die uns für eine experimentelle Bearbeitung dieser Frage einige Ansatzpunkte zu bieten schienen.

Den Anstoss dazu gab eine ältere Mitteilung von RADEMACHER u. SCHMIDT (1933), nach der speziel von Rivanol (3,9-Diamino 7-äthoxyacridin) ein starker Schlüpfreiz auf die enzystierten Larven von *Heterodera schachtii* ausgeht. Da die Gruppe der Aminoacridine heute von physiologisch-pharmakologischer Seite besonderes Interesse gefunden hat (vgl. WAGNER-JAUREGG, 1936 u. 1949), hielten wir es für geraten, diese in der Natur nicht vorkommende Substanz in ihrer Wirkung etwas eingehender zu untersuchen. In einigen Vorversuchen konnten wir zunächst folgende Beobachtungen machen:

1. Rivanol wirkt, trotz seiner stark bakteriziden Eigenschaften, auch in gesättigter Lösung nicht schlüpfhemmend auf die enzystierten Larven; d.h. ein negativer Wirkungsflügel dieser Präparates ist praktisch nicht nachweisbar.

*) Eingegangen: 26. Oktober 1956.

2. Auch bereits geschlüpfte, aber schon weitgehend inaktive Larven werden durch Rivanol stimuliert und zu verstärkter Bewegung angeregt.
3. Rivanol übt einen Richtungsreiz aus; (Nachweis durch Haltung der Würmer in Agar und Beobachtung der Wanderungsbewegungen, RENSCH, 1925).

Es war demnach zu vermuten, dass es sich bei der Wirkung dieser Substanz entweder um einen Sinnesreiz auf die schlüpfbereiten Larven oder — möglicherweise in Verbindung damit — um eine spezifische Wirkung auf die Stoffwechselvorgänge des Respirationsapparates und der locomotorischen Erfolgsorgane handelt.

Die meisten bisher durchgeführten biologischen Untersuchungen an Acridinderivaten befassen sich ausschliesslich mit der antiseptischen Wirkung von Aminoacridinen und gehen weniger auf grundsätzliche physiologische Fragen ein. In seiner umfassenden Darstellung der Synthese und pharmakologischen Eigenschaften der Acridine betont ALBERT (1951) insbesondere die mögliche Bindung basischer Acridinionen an negative Molekülgruppen mit spezifischer Funktion in den Zellmembranen, wobei vermutlich auch die Sorbierbarkeit des jeweiligen Acridinmoleküls (van der WAALS'sche Kräfte) entsprechend seiner sterischen Konfiguration eine Rolle spielt. Auch der Oberflächenaktivität mancher Acridinderivate (z.B. Rivanol) kommt möglicherweise eine gewisse Bedeutung für ihre biologische Wirksamkeit zu. Gleichzeitig werden jedoch auch Beobachtungen angeführt, die diesen Vorstellungen nicht ganz entsprechen.

Wir hielten es daher für angebracht, diese Frage am Beispiel der Larvenaktivierung bei *Heterodera schachtii* nachzuprüfen durch einen Vergleich von zwei Aminoacridinen, die — bezogen auf die Grenzfläche Wasser: Luft — einmal durch eine sehr grosse (3,9-Diamino-7-äthoxyacridin) und zum andern durch eine sehr geringe Oberflächenaktivität (9-Aminoacridin) gekennzeichnet sind [1]).

Gleichzeitig sollte mit einer Erweiterung dieses Versuches eine zweite Frage beantwortet werden. Ausgangspunkt für unsere Überlegungen war die bekannte Sensibilität der Nematoden gegenüber oxydierenden Substanzen und solchen Körpern, die geeignet sind „vitale Oxydationen" auszulösen, ohne dass dabei an eine unmittelbare Sauerstoffwirkung gedacht werden muss (RENSCH 1925).

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1) Herabsetzung der Oberflächenspannung von Wasser bei 15° C und pH 7 bei 2,5 × 10.4 mol/l beträgt für 3,9-Diamino-7-äthoxyacridin 12,5 dyn/cm und für 9-Aminoacridin 0 dyn/cm (ALBERT, 1951).

Breyer (1944) und Mitarbeiter konnten nämlich polarographisch feststellen, dass eine deutliche Parallele besteht zwischen dem Reduktionspotential der einzelnen Aminoacridine und ihrer biologischen Wirksamkeit. Die Verbindungen mit dem am stärksten negativen Reduktionspotential (9-Aminoacridin und 3,6-Diaminoacridin) sind durch den höchsten bakteriostatischen Index gekennzeichnet. Es wurde aufgrund dieser Ergebnisse von den Autoren die Vermutung ausgesprochen, dass es sich bei der Wirkung dieser Substanzen vielleicht um einen Eingriff in das enzymatische Geschehen im Atmungssystem der Bakterien handelt, der durch die reduktiven Kräfte des Organismus nicht mehr korrigiert werden kann und daher irreversibel wird.

Da aufgrund dieser Arbeit die Werte für die Redoxpotentiale verschiedener Aminoacridine bereits vorlagen und einige dieser Substanzen in ihrer Konstitution dem Rivanol nahe verwandt sind, sollte in Vergleichsversuchen geprüft werden, ob eine dieser Theorien für eine Erklärung der von Rivanol ausgehenden starken Reizwirkung bei *Heterodera schachtii* herangezogen werden kann. Es ergibt sich nämlich unter diesem Gesichtspunkt auch ein entfernter Zusammenhang mit der Frage nach dem natürlichen Schlüpfreiz, da neuerdings Geller (1953) nachgewiesen hat, dass durch Wurzelausscheidungen von Pflanzen das Redoxpotential des Bodens in der Rhizosphäre verändert wird.

Aus Tabelle 1 ist ersichtlich, welche Verbindungen auf ihre reizphysiologische Wirkung untersucht wurden [1]). Die Versuchsergebnisse decken sich weitgehend mit einem anderen Kontrollversuch, der mit einer besonders schlüpfbereiten Zystenreihe durchgeführt wurde.

Aus den Versuchen lassen sich folgende Schlüsse ziehen:

1. Die stark schlüpfreizende Wirkung des Rivanols kann — wie nach den vorausgegangenen Untersuchungen (s.o.) zu vermuten war — durch Veränderungen der Oberflächenspannung an den Grenzflächen von wässriger Phase und Zellwandung (Eihülle, Larvenkutikula) nicht erklärt werden. So setzt zum Beispiel das dem Rivanol sehr ähnliche 9-Aminoacridin die Oberflächenspannung des

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1) Methode des Versuches in Anlehnung an Rademacher (1930): 3 × 20 volle, vorgewässerte Zysten je Versuchsglied. Auszählung im zweitägigen Abstand. Larvenzahl (S_{b1}) = Summe von 3 Auszählungen. Einwirkungszeit der Testlösung: 4 Tage. Über Einzelheiten der Versuchsmethodik wird in einer späteren Veröffentlichung berichtet.

Für die freundliche Überlassung der benötigten Acridine sei den Farbwerken Hoechst und den Bayer-Werken in Leverkusen besonders gedankt.

Wassers fast gar nicht herab, wirkt aber im biologischen Test von allen übrigen Acridinderivaten am stärksten schlüpfreizend.

TABELLE 1

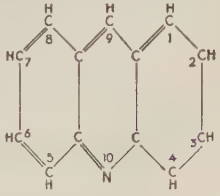
Die aktivierende Wirkung von Aminoacridinen

Versuchsglied Nr.	Substanz [1] (0,0001 Mol/1)	S_{bl}	RE_u
1	Acridin	65	1,48
2	3,6-Diaminoacridin	83	1,89
3	3,6-Tetramethyldiaminoacridin	50	1,14
4	Chlormethylat des 3,6-Diaminoacridins	132	3,00
5	3,6-Diacetaminoacridin	95	2,16
6	5,9-Diaminoacridin	56	1,27
7	5-Nitro-9-Aminoacridin	49	1,11
8	5-Methoxy-9-Aminoacridin	57	1,30
9	6-Chlor-9-Aminoacridin	187	4,25
10	7-Methoxy-9-Aminoacridin	232	5,27
11	9-Aminoacridin	395	8,98
12	Atebrin	57	1,30
13	Rivanol (3,9-Diamino-7-äthoxyacridin)	438	9,95
14	Unbehandelt	44	1,00

S_{bl} = Zahl der geschlüpften Larven / Versuchsglied (3 Wiederholungen)
RE_u = Reizeffekt bezogen auf „Unbehandelt" (Unbehandelt = 1)

2. Möglicherweise an der Eimembran eintretende Veränderungen (vgl. NOLTE 1956) sind für die eigentliche „Aktivierung" der Larven durch Rivanol ohne Bedeutung.
3. Es ist nicht möglich, die unterschiedliche Reizwirkung einzelner Aminoacridine in Beziehung zu ihrem Redoxpotential zu setzen oder dadurch zu erklären. Von zwei Aminoacridinen mit ausgesprochen stark negativem Redoxpotential wirkt die eine auf die enzystierten Larven sehr schlüpfreizend (9-Aminoacridin; Red.-pot.: -0,916 Volt) während die andere (3,6-Diaminoacridin; Red.-pot.:-0,731 Volt) gar keinen Reiz ausübt. Eine „oxydative" Wirkung der betreffenden Aminoacridine kann demnach nicht zur Erklärung des Wirkungsmechanismus herangezogen werden, zumal

1) Der Bezifferung des Acridinkernes (s. Abb.) liegt die in Deutschland gebräuchliche Nomenklatur zugrunde (vgl. KARRER, Lehrbuch der organischen Chemie, 1954). — Die unter Nr. 1 u. Nr. 5-7 aufgeführten Substanzen wurden wegen ihrer geringen Wasserlöslichkeit nicht in der angegebtnen Konzentration, sondern in gesättigter Lösung getestet.



ACHIDIN

man dann erwarten müsste, dass in erster Linie Substanzen, die sich durch ein positives Redoxpotential auszeichnen, den Schlüpfprozess begünstigen.

4. Die Reizwirkung ist nicht an eine C-9-Stellung der Aminogruppe gebunden. So ist z.B. 5-Methoxy-9-Aminoacridin praktisch unwirksam, während von 3,6-Diamino-10-Chlormethylacridin ein nicht sehr starker, aber deutlicher Schlüpfreiz ausgeht.

Ein generelles Prinzip für den vermutlichen Wirkungsmechanismus bei der Reizperzeption und Reizbeantwortung lässt sich aus den hier aufgeführten Versuchen und aus orientierenden Experimenten mit anderen pharmakologischen Erregungssubstanzen bisher nicht erkennen.

SUMMARY

Some amino-acridines were investigated for their hatching activity on eggs of *Heterodera schachtii*. Stimulation of *Heterodera* larvae within the cysts by some of the substances tested cannot be explained by the surface-activity or redox-potentials of the compounds. The stimulating effect is not restricted to 9-amino-acridines. Results are discussed in regard to the possible mechanism of activation by chemical stimulus.

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(*Nematologica* II (1957): 131-143)

BEZIEHUNGEN ZWISCHEN WIRT UND PARASIT IM INFEKTIONSVERLAUF VON *HETERODERA SCHACHTII* SCHMIDT IN KURZFRISTIGEN TOPFVERSUCHEN

I. INFEKTIONSGANG BEI VERSCHIEDENEN WIRTSPFLANZEN [1])

VON

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Bei der Laborprüfung von neuen Nematiziden, die den Charakter einer orientierenden Vorprüfung trägt, werden vielfach Topfversuche durchgeführt. Diese Versuche erfolgen hier in Neubauer-Schalen (h = 6 cm, ø = 11 cm), die mit einer konstanten Menge von 380 ccm rüben- bzw. kartoffelnematodenverseuchter Erde gefüllt sind und in die nach Beimischung des Mittels die Wirtspflanzen eingesät werden. Die Temperatur beträgt bei diesen Versuchen + 20° C. Wegen der gleichmässigen Infektionsfähigkeit werden sehr häufig Rübennematoden als Testtiere benutzt. Ausserdem sind die für *Heterodera schachtii* Schm. brauchbaren Wirtspflanzen (Rübsen, Zuckerrüben, Raps oder Senf) schneller wüchsig als beispielsweise Kartoffeln oder Tomaten. Die schnellere Entwicklung schliesst auch das Wachstum des Wurzelsystems ein, das damit dem Parasiten bereits nach kurzer Zeit günstige Einwanderungsmöglichkeiten bietet. Aus diesem Grunde können die Versuche nach verhältnismässig kurzer Zeit abgebrochen werden. Beim Rübennematoden sehen wir 21 Tage nach Einsaat der Wirtspflanzen als ausreichend an, wobei nach 12, 16 und 21 Tagen die Kontrolle der Wurzeln auf eingewanderte Larven und deren weitere Entwicklungsstadien erfolgt. Auf Einzelheiten der Testmethodik zur Mittelprüfung soll jedoch hier nicht weiter eingegangen werden.

Bei den Laborprüfungen zeigte es sich wiederholt, dass bestimmte Nematizidproben offenbar den Gang der normalen Infektion erheblich verändern können, etwa in Richtung einer Förderung des Nebenwurzelbefalls, einer Verzögerung des Auftretens bestimmter Entwicklungsstadien oder in Hinblick auf eine Änderung des Geschlechtsverhältnisses. Aus diesem Grunde schien es wünschenswert, den normalen Infektionsverlauf zunächst für *Heterodera schachtii* Schm. festzule-

1) Eingegangen: 9. September 1956.

gen, wobei ebenfalls ein Zeitabschnitt von 21 Tagen zugrunde gelegt wurde. Ausserdem sollte geprüft werden, welche der üblicherweise benutzten Wirtspflanzen von *H. schachtii* Schm. die höchsten Einwanderungszahlen aufzuweisen habe und damit für einen Vergleich behandelter und unbehandelter Töpfe am günstigsten sei. Schliesslich liessen sich bei dieser Gelegenheit einige biologische Daten, die mit dem Infektionsmodus in Verbindung stehen, überprüfen.

Methode. Die zur Füllung der Neubauer-Schalen benutzte Erde wies eine Verseuchung von durchschnittlich 95 Zysten pro 100 ccm Boden auf. Jede Schale enthielt 380 ccm dieser Erde und wurde mit jeweils 40 Samen bzw. bei Rüben 30 Knäuel, die etwa 1 cm tief eingebracht waren, versehen. Die Versuchstemperatur betrug + 20° C, die Luftfeuchtigkeit 70 % rel. Feuchte. Die Topferde wurde gleichmässig mit Wasser versehen.

Jede Reihe bestand aus 10 Töpfen, aus denen bei jeder Kontrolle 3 Pflanzen entnommen wurden. Damit kamen bei jeder Kontrolle die Wurzeln von je 30 Pflanzen zur Untersuchung. Die Wurzeln mussten vorsichtig ausgegraben und mit einem Haarpinsel von anhaftenden Bodenteilchen gereinigt werden. Durch Färbung mit Jodjodkalium und gegebenenfalls Differenzierung mit Alkohol und leichtes Pressen der Wurzeln zwischen 2 Objektträgern konnten die eingewanderten Tiere leicht ausgezählt und ihr Entwicklungszustand sowie ihre Lagerichtung bestimmt werden. Schliesslich wurde die Wurzellänge, getrennt in Haupt- und Nebenwurzeln, sowie ihr Durchmesser festgehalten. Bei komplizierten Wurzelsystemen erfolgte eine fotografische Aufnahme, mit deren Hilfe die Länge von Haupt- und Nebenwurzeln genau festgelegt werden konnte.

Als Entwicklungsstadien unterschieden wir folgende:

1. Larve II als das in die Wurzel einwandernde Stadium.
2. Larve III, nach der ersten in der Wurzel erfolgten Häutung. Sie ist etwas kürzer und dicker als die Larve II.
3. Larve IV ist flaschenförmig und hat ihre wurmförmige Gestalt verloren.
4. Juvenile Weibchen bzw. Männchen, wobei auch die Männchen, die sich noch in der letzten Larvenhülle befanden, als solche gezählt wurden, da diese klar als männliche Tiere zu erkennen sind.

Die Kontrollen fanden in den ersten 6 Tagen alle 24 Stunden statt, von da ab nur alle 3 Tage. Es wurden nur frisch und gesund aussehende Pflanzen zur Kontrolle herangezogen, da Larven II aus kran-

ken Wurzeln abwandern könnten. Bei der Festlegung der Kopfrichtung sind nur Tiere gewertet, deren Kopf- und Körperlage klar erkannt werden konnte, die übrigen wurden als fraglich registriert.

Im weiteren ist als absoluter Befall die Zahl der eingewanderten Stadien pro Wurzelsystem einer Pflanze bezeichnet, während als Befallsdichte die Zahl der Tiere pro 10 cm Wurzellänge wiedergegeben ist. Die in den Kurven dargestellten Werte stellen Durchschnittswerte aus je 30 untersuchten Wurzelsystemen dar.

INFEKTION VERSCHIEDENER WIRTSPFLANZEN

1. Zuckerrüben (*Beta vulgaris L. f. altissima D.C.*, Sugar beet)

Bei Benutzung von Zuckerrüben als Versuchspflanzen zeigte es sich, dass die ersten sichtbaren Wurzelbildungen bereits am 2. Tage nach Aussaat festzustellen waren. Damit ist praktisch auch die Möglichkeit für einsetzenden Befall durch Larven II gegeben. Wie aus Abb. 1 zu entnehmen ist, tritt tatsächlich schon nach 3 Tagen der erste Befall auf. Die Einwanderung von Larven setzt aber erst einen Tag später in vollem Umfang ein. Das Maximum des absoluten Befalls pro Pflanze ist am 18. Tage erreicht. Bei Betrachtung der Kurve ergibt sich am 15. Tage ein auffallendes Absinken des Gesamtbefalls, das — wie die Kurve der Wurzellängen zeigt — mit einem entsprechenden Ansteigen der jeweiligen Wurzellänge gekoppelt ist. Diese Diskrepanz ergibt sich aus der Schwierigkeit, dass bei jeder Kontrolle ein anderes Wurzelmaterial zur Prüfung herangezogen werden musste. Die den Töpfen entnommenen Wurzeln müssen gereinigt und gemessen und schliesslich angefärbt werden, alles Vorgänge, die eine Weiterentwicklung dieser Pflanzenteile und ebenso der eingewanderten Larven unmöglich machen. Die Beseitigung dieser Schwierigkeit liesse sich nur durch eine weitgehende Vergrösserung des Versuchsmaterials bis zu einem gewissen Grade erreichen.

Die wohl zufällig so starke Vergrösserung der Wurzellänge ruft den Knick in der Kurve des Gesamtbefalls hervor. Zumindest die Kurve der Wurzellängen muss eine ansteigende Tendenz haben, denn die Wurzellängen können mit zunehmendem Wachstum schwerlich geringer werden.

Wie nicht anders zu erwarten, liegt das Auftreten der verschiedenen Larvenstadien in zeitlicher Staffelung hintereinander. Bereits nach 6 Tagen treten die ersten Larven III, nach 9 Tagen Larven IV und

etwa nach 15 Tagen die ersten juvenilen Männchen und Weibchen auf. Bemerkenswert ist es weiter, dass die Zahl der eingewanderten Larven II zwischen 6 und 9 Tagen Höchstwerte aufweist, die während der hier gewählten Versuchsdauer nur annähernd am 18. Tage wieder erreicht werden, also etwa zu einem Zeitpunkt, an dem die ersten voll

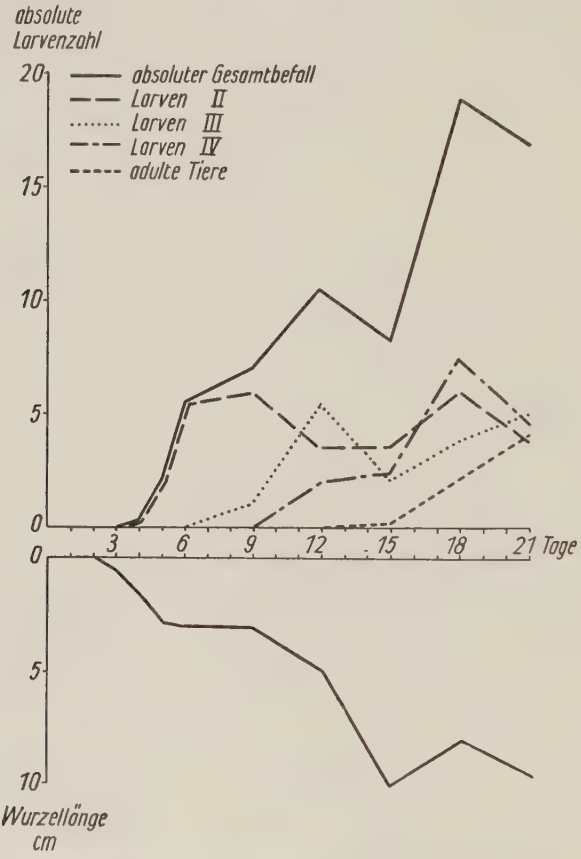

Abb. 1: Wurzellänge, Gesamtbefall und Auftreten der einzelnen Entwicklungsstadien von Heterodera schachtii Schm. für Zuckerrüben als Wirtspflanze.

entwickelten Tiere in der Wurzel auftreten. Schon in anderen Versuchen war der Eindruck einer stossweisen Einwanderung der Larven II entstanden, wobei eine neue Einwanderungswelle immer dann auftritt, wenn sich die Spitze der vorhergehenden Welle bereits der Vollendung ihrer Entwicklung nähert. Eine eingehende Untersuchung dieser interessanten Spezialfrage erscheint lohnend und ist vorgesehen.

Die Kurve für die Larven III weist am 15. Tage ein deutliches Absinken auf. Dieser Rückgang ist wesentlich mit verantwortlich für den Tiefstand der Kurve des Gesamtbefalls, auf den oben bereits hingewiesen wurde. Es wird später nochmals auf dieses Absinken der Zahl der Larven III einzugehen sein.

2. Rübsen (*Brassica rapa L. v. silvestris* (Lam.) Purch. et Ley, Turnip rape).

Die im Versuch benutzten Sommerrübsen weisen bereits nach 2 Tagen die ersten erkennbaren Wurzelbildungen auf. Das Wurzelwachstum verläuft dann verhältnismässig stetig, hält sich aber hinsichtlich der Gesamtlänge in recht geringen Grenzen. Das Auftreten der einzelnen Stadien und ihre weitere Entwicklung ist aus Abb. 2 zu ent-

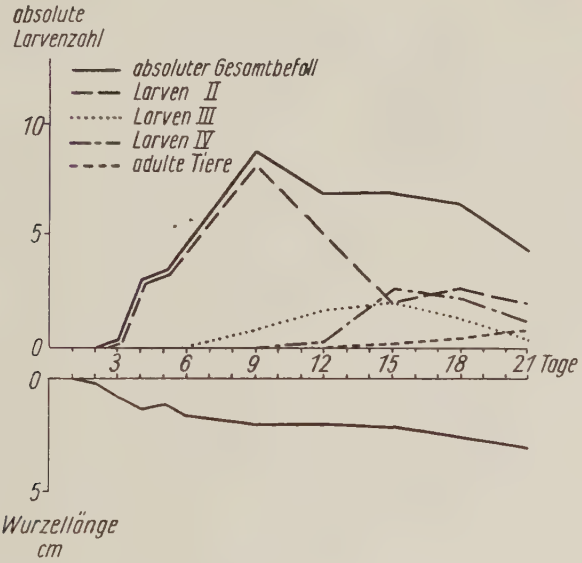

Abb. 2: Wurzellänge, Gesamtbefall und Auftreten der einzelnen Entwicklungsstadien von Heterodera schachtii Schm. für Rübsen als Wirtspflanze.

nehmen. Dabei zeigt es sich, dass das Auftreten der einzelnen Stadien in den Wurzeln etwa zum gleichen Zeitpunkt erfolgt wie bei den Zuckerrüben. Abweichend ist jedoch die frühe Lage des Kurvengipfels für den Gesamtbefall, der hier bereits nach 9 Tagen auftritt. Das anschliessende Abfallen der Kurve ist im wesentlichen durch die deutlich geringer werdende Zahl der weiter einwandernden Larven

bestimmt, ausserdem aber durch den verhältnismässig geringen Anteil, den die Larven III am Gesamtbefall haben. Es hat damit den Anschein, dass sich bei weitem nicht alle Larven II bis zur Larve III entwickelt haben. Der Tiefpunkt für die Zahl der Larven II liegt in diesem Versuch an einem Zeitpunkt, an dem auch die ersten erwachsenen Stadien auftreten. Der anschliessende Anstieg der Zahl der Larven II ist jedoch nur äusserst gering.

3. Senf (*Sinapis alba* L., White mustard).

Das Wurzelwachstum der Senfpflanzen erweist sich in diesem Versuch als ausgeglichen und zeigt eine stetige Zunahme. Es ist im ganzen

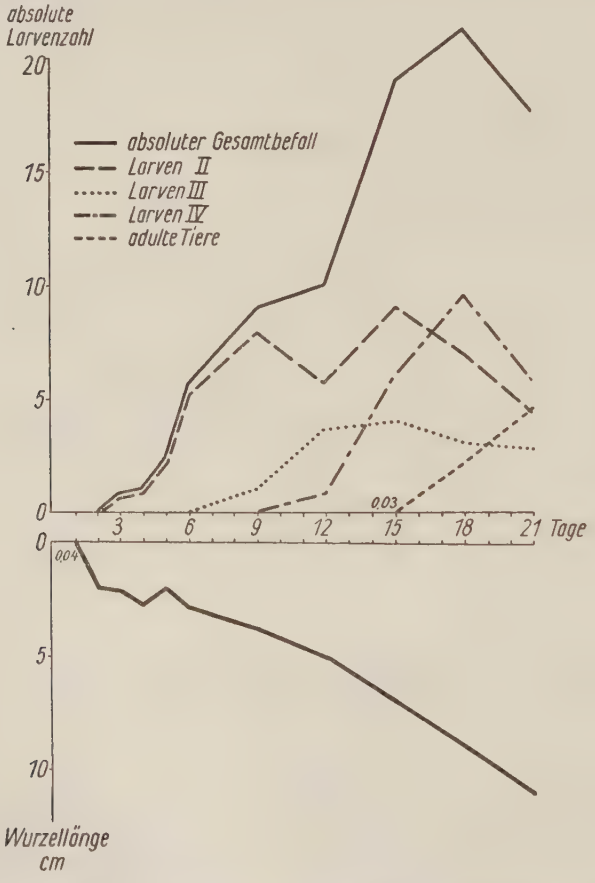

Abb. 3: Wurzellänge, Gesamtbefall und Auftreten der einzelnen Entwicklungsstadien von Heterodera schachtii Schm. für Senf als Wirtspflanze.

kräftiger als bei den Rübsen, verläuft aber etwa in der Weise wie bei Zuckerrüben. Die Gesamtbefallskurve (Abb. 3) erreicht am 18. Tage den Höchstwert. Die Haupteinwanderung der Larven II setzt etwa am 4. Tage ein; ihre Zahl steigt bis zum 9. Tag an. Ab 15. Tage fällt die Menge der in den Wurzeln vorhandenen Larven II wieder, liegt aber im Vergleich zum entsprechenden Versuch mit Rübsen noch verhältnismässig hoch, wie überhaupt die Kurve des Gesamtbefalls bei Senf wesentlich höher liegt als bei Rübsen. Als ein Grund dürfte das höhere Angebot an Wurzelmasse bei Senf angesehen werden.

Das Auftreten der einzelnen Larvenstadien des Nematoden erfolgt zu den gleichen Zeitpunkten wie bei den anderen Wirtspflanzen auch. Das Missverhältnis zwischen Zahl der Larven III und IV dürfte seine Erklärung wiederum in der Untersuchungsmöglichkeit finden, die durch Benutzung immer anderen Materials nur relativ vergleichbare Werte ergibt.

4. Raps (*Brassica napus* L. *v. arvensis* (Lam.) Thell., Swede rape).

Die Wurzelentwicklung setzt hier bereits nach einem Tag ein und zeigt eine stetige, allmähliche Zunahme. Die Entwicklung des Wurzelsystems entspricht weitgehend der für Rüben und Senf. Der kennzeichnendste Unterschied zu den übrigen Kurvendarstellungen liegt bei der Wiedergabe dieses Versuches (Abb. 4) in der relativ hohen Lage der Kurve für den Gesamtbefall. Dieser steigt praktisch bis zum Versuchsende an, wobei von dem Knick am 18. Versuchstag abgesehen werden soll. Unter Zugrundelegung der später zu behandelnden Befallsdichte (Zahl der Nematoden pro 10 cm Wurzellänge) ist ein gleichmässiges Ansteigen des Gesamtbefalls anzunehmen.

Die Einwanderung der Larven II, die mit dem 3. Tage einsetzt, erreicht zwischen dem 12. und 15. Tag ein Maximum; sie sinkt dann gegen Versuchsende deutlich ab, hier also wieder etwa zu einem Zeitpunkt, an dem die ersten adulten Tiere auftreten. Annähernd die gleiche Höhe wie die Zahl der Larven II erreichen auch — natürlich mit einer entsprechenden zeitlichen Verschiebung — die Larven III, während die Larven IV ab 18. Versuchstag ihre Maximalwerte aufweisen. Zu diesem Zeitpunkt setzt auch bereits ein steiler Anstieg der Zahl adulter Tiere ein.

Die relativ grosse Zahl aller Entwicklungsstadien bedingt letzten Endes auch die bis zum Versuchsende ansteigende, hohe Lage der Gesamtbefallskurve.

Schon HORNBURG (1928) hatte die unterschiedliche Befallsstärke für Rüben, Rübsen und Senf festgestellt, wobei Rüben den höchsten absoluten Befall aufwiesen. Die Versuche HORNBURGS lassen sich jedoch aus verschiedenen Gründen nicht unmittelbar vergleichen.

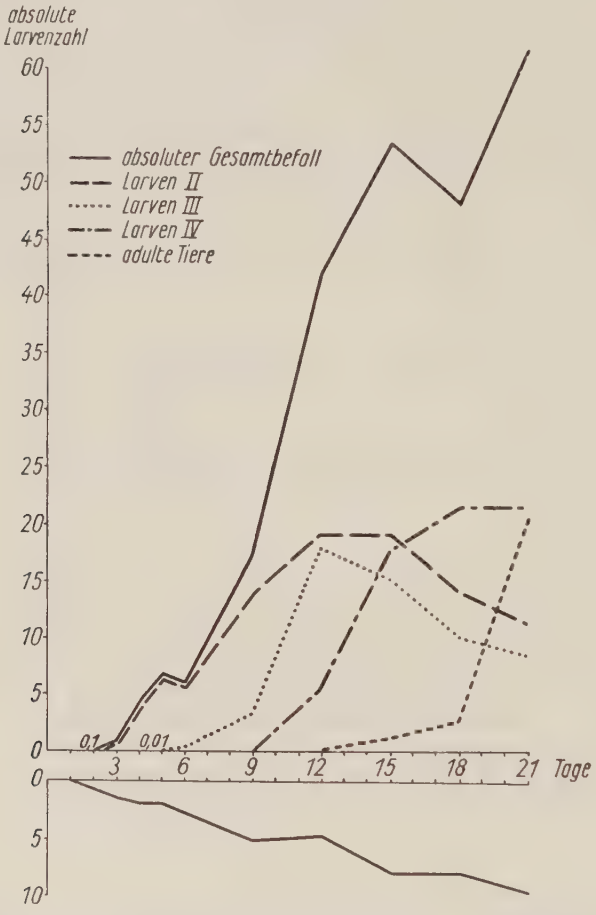

Abb. 4: Wurzellänge, Gesamtbefall und Auftreten der einzelnen Entwicklungsstadien von Heterodera schachtii SCHM. für Raps als Wirtspflanze.

Bei Betrachtung der Wurzellängen der hier geprüften Pflanzenarten ist zunächst festzustellen, dass Rübsen die geringste Wurzelentwicklung aufweisen. Dabei ist stets die Gesamtlänge des Wurzelsystems zugrunde gelegt. Die übrigen 3 Wirtspflanzen weichen nicht wesentlich in ihrer Wurzelentwicklung voneinander ab. Der Vergleich der Wur-

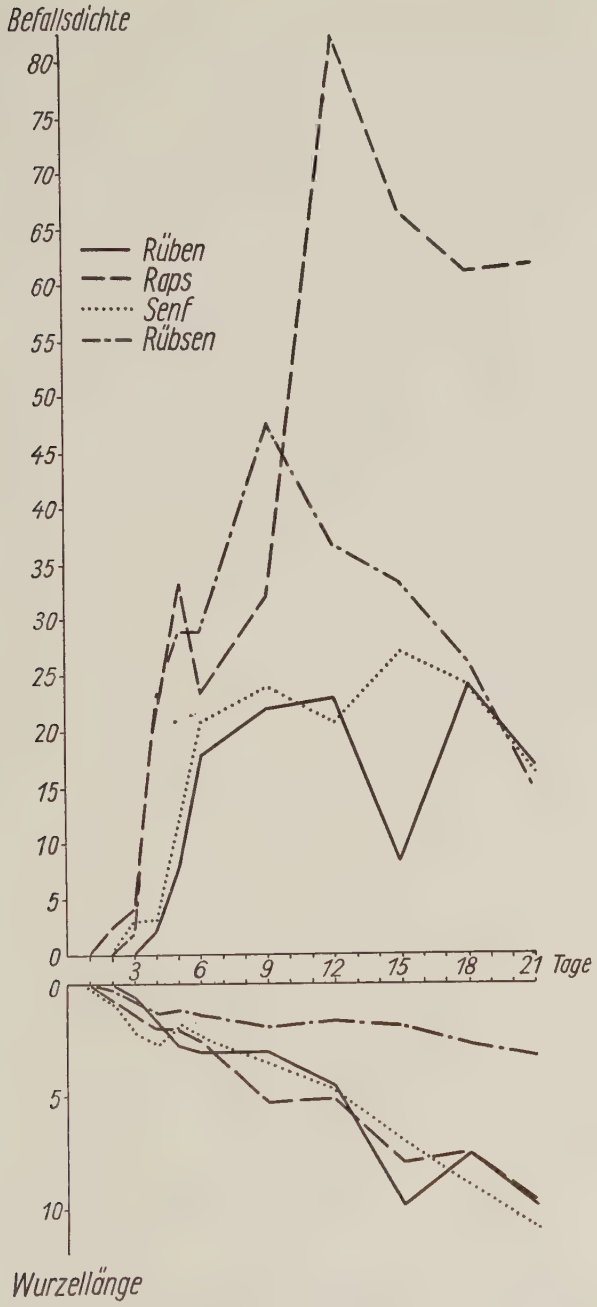

Abb. 5: Wurzellänge und Befallsdichte (Larven pro 10 cm Wurzellänge) von Heterodera schachtii Schm. für verschiedene Wirtspflanzen.

zelentwicklung ist für die Betrachtung der Befallszahlen insofern wichtig, als mit stärkerem Wurzelwachstum auch mehr Attraktivstoffe auf die einwandernden Larven wirken und weiter eine grössere räumliche „Aufnahmekapazität" für die Parasiten vorhanden ist. Die Befallsdichte, also die Zahl der Stadien pro 10 cm Wurzellänge, führt hier zu interessanten Vergleichen (Abb. 5). Diese Darstellungsweise zeigt, welche Wirtspflanze und welcher Zeitpunkt die grösste Dichte an eingewanderten Stadien aufzuweisen hat. Die höchste Dichte erreicht das Rübenälchen in Rapswurzeln; die grösste Dichte im Befall geht mit dem höchsten absoluten Befall der Wirtswurzeln an Larven II zeitlich konform. Der Gipfel der Befallsdichte liegt bei 12 Tagen, während der absolute Befall an Nematoden bis zum Versuchsende weiter ansteigt. Daraus ergibt sich, dass durch das ansteigende Wurzelwachstum eine Auflockerung der Besiedlungsdichte im Wurzelsystem erfolgt und dass gleichzeitig auch diese Auflockerung durch ein Nachlassen der Einwanderung neuer Larven II gefördert wird. Die hohe Besiedlungsdichte spricht für eine optimale Ausnutzung des gebotenen Lebensraumes in der Rapswurzel durch den Nematoden. Anders ausgedrückt ist der Minimallebensraum des Parasiten — um diesen ökologischen Begriff zu benutzen — in Rapswurzeln absolut gesehen kleiner als bei den übrigen geprüften Wirtspflanzen.

Die nächsthöhere Befallsdichte erreicht das Rübenälchen in Rübsenwurzeln. Bezogen auf die relativ kleinen Wurzellängen ist der absolute Befall ziemlich hoch, so dass sich auch eine relativ hohe Befallsdichte ergibt. Ebenso wie auch für alle anderen Wirtspflanzen ist der Anstieg der Besiedlungsdichte in den ersten 6 Tagen besonders steil. Der Gipfel ist für Rübsen bereits am 9. Tage erreicht. An diesem Tage liegt auch der höchste absolute Befall, und die Larven II in der Wurzel weisen am gleichen Tage ihr Maximum in den Wurzeln auf. Ihre Zahl geht dann auffallend stark zurück, so dass es dann nicht mehr zu einer so dichten Besiedlung des Wurzelsystems kommen kann, wie sie am 9. Tage erreicht war. Infolge des verhältnismässig schwachen Wachstums der Rübsenwurzeln kann die Auflockerung der Befallsdichte weniger auf Wurzelwachstum zurückgeführt werden als auf das starke Absinken der Einwanderung nach dem 9. Tage. Wie Abb. 2 zeigt, liegt auch die Zahl der älteren Entwicklungsstadien des Parasiten ziemlich tief, was darauf hindeuten könnte, dass die Entwicklungsbedingungen in den Rübsenwurzeln besonders für die älteren Larvenstadien trotz anfänglich guter Einwanderung nicht sehr günstig sind. Die Befallsdichte in Rüben und Senf verhält sich etwa bis zum 12. Tage recht

ähnlich. Ein Maximalwert, wie er bei den zuerst besprochenen Wirtspflanzen zu erkennen war, ist hier kaum ausgeprägt. Wenn er zwischen den einzelnen Kontrollterminen vorhanden sein sollte, dann wäre er zwischen dem 9. und 15. Tage zu suchen. Deutlich ist das Absinken der Befallsdichte gegen Versuchsende. Das auffallende Minimum für Rüben am 15. Tage dürfte auf die Unterschiedlichkeit des geprüften Wurzelmaterials zurückzuführen sein, da an diesem Tage eine relativ grosse Wurzellänge gemessen wurde, die mit geringem Befall einherging. Die grösste Befallsdichte fällt in Zuckerrübenwurzeln auch mit dem höchsten absoluten Befall zusammen. Dieser wieder ist die Folge einer ansteigenden Zahl von Larven II, die mit einer grösseren Zahl schon älterer Larven in den Wurzeln zusammentrifft.

Für Senf liegt der höchste absolute Befall 3 Tage später als die grösste Befallsdichte, die am 15. Tage auftritt. Die grösste Dichte des Wurzelbefalles entsteht wiederum als Folge einer grossen Zahl eingewanderter Larven II und hier einer starken Zunahme der Larven IV in den Wurzeln. Es scheint so, als ob die Larven der ersten Einwanderungswelle sich sehr schnell in Larven IV umwandeln, so dass das Stadium III nur kurzfristig durchlaufen wird. Damit könnte sich die relativ flach verlaufende Kurve für die Larven III in Abb. 3 erklären.

Zusammenfassend kann festgestellt werden, dass Raps hinsichtlich des absoluten Befalles und der Befallsdichte durch *H. schachtii* Schm. am günstigsten liegt und damit als Wirtspflanze für Topfversuche zur Mittelprüfung am besten geeignet erscheint. Es verhalten sich die durchschnittlichen absoluten Zahlen der eingewanderten Stadien über die gesamte Versuchsdauer für Raps, Senf, Rüben und Rübsen wie 100 : 37 : 29 : 19, die mittlere Befallsdichte in der gleichen Reihenfolge wie 100 : 39 : 33 : 61.

Die grösste Befallsdichte liegt im allgemeinen früher als die grösste absolute Dichte. Sie tritt ein, wenn 3 Faktoren eine günstige Konstellation zueinander erreicht haben, nämlich 1. das Wurzelsystem noch relativ klein ist und damit eine Auflockerung der Befallsdichte noch nicht eintreten konnte; 2. die Zahl der eben eingewanderten Larven II, die im allgemeinen nach einer Einwanderungsperiode wieder zurückgeht, noch relativ hoch liegt und 3. die am frühesten eingewanderten Larven sich bereits in grösserer Zahl in Umwandlung zu späteren Stadien befinden.

Auch der Prozentsatz der Larven III, die sich aus den eingewanderten Larven II zu entwickeln vermochten, weist für Raps die günstigste Lage auf:

Raps	57,4 %
Zuckerrüben	56,9 %
Weisser Senf	32,4 %
Sommerrübsen	19,4 %.

Hierbei ist jedoch die relativ kurze Versuchsdauer und der Umstand, dass jeweils immer andere Wurzeln untersucht werden mussten, zu berücksichtigen, so dass die Werte nur Anhalt sein können.

Schliesslich ist bei der Bewertung unterschiedlichen Befallsverhaltens gegenüber den verschiedenen Wirtspflanzen auch die unterschiedliche physiologische Tendenz des Nematoden zu berücksichtigen, die sich aus einer mehr oder weniger langen Gewöhnung an eine bestimmte Wirtspflanze entwickelt haben könnte. In unserem Falle war das Versuchsmaterial mehrere Generationen lang an Zuckerrüben vermehrt worden. Die Frage der spezialisierten Stämme, die schon mehrfach aufgeworfen worden ist (Baunacke, 1922; Franklin, 1940; Goffart, 1926, 1928, 1931, 1949; Steiner, 1925; u.a.), soll hier nicht näher diskutiert werden. Das Bestehen solcher spezialisierten Stämme könnte bei ähnlichen Versuchen möglicherweise zu merklichen Unterschieden in den Befunden führen.

SUMMARY

The normal course of invasion and development of beet eelworm in sugar beet, summer turnip rape (Sommerrübsen), swede rape (Raps) and white mustard was studied during the first 21 days of plant growth. The number of larvae in the whole root system was greatest in swede rape followed by white mustard, beet and turnip rape. There were no appreciable differences in the time of appearance of the different larval stages in the different host plants. The density of larvae per 10 cm. of root length was again highest in swede rape followed by turnip rape, mustard and beet. Among other things which contribute to a high larval density is relatively slow growth in root length. The percentage of larvae in stage III which dveloped from larvae invading in stage II was greatest in the roots of swede rape and beet.

Swede rape therefore appears to be the best host plant amongst those tested and is likely to be the most useful in pot tests.

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RADOPHOLUS LAVABRI N.SP. (NEMATODA, TYLENCHIDAE) PARASITE DU RIZ AU CAMEROUN FRANÇAIS [1])

PAR

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En Octobre 1955, nous avons reçu de la part de M. E. Lavabre, Entomologiste dépendant du Service de l'Agriculture au Cameroun, un échantillon de racines de riz âgé de 2 à 3 mois provenant de la région de Koko (subdivision de Yagoua-Nord Cameroun), en bordure du Logone. M. E. Lavabre nous signalait, dans une lettre accompagnant l'envoi, que les rizières de cette région présentaient des zones plus ou moins étendues où les pieds de riz étaient étiolés, jaunissants, tout en ayant la même taille que les pieds sains. Dans certains cas les insectes étaient responsables des dégâts mais dans beaucoup d'autres aucun insecte n'est présent.

C'est dans des racines provenant de ce dernier type d'affection que nous avons trouvé de très nombreux juvéniles et quelques adultes d'un *Radopholus*. Les individus sont entièrement logés dans les tissus radiculaires, allongés lorsqu'il s'agit de formes jeunes, enroulés sur eux — mêmes dans le cas de juvéniles aux derniers stades et d'adultes; les individus et les oeufs sont isolés. On retrouve cette même espèce dans le sol entourant les racines et entre les bases des gaines foliaires.

L'espèce trouvée était assez semblable morphologiquement à *Radopholus oryzae* (v. Breda de Haan, 1902), Thorne 1949, mais en différait par des dimensions nettement supérieures. La même année étaient publiés deux travaux, l'un de I. Andrassy (1954) décrivant *Radopholus gigas,* espèce voisine de *R. oryzae,* mais beaucoup plus grande, trouvée associée aux racines d'un „lys d'eau" en Roumanie, l'autre de H. Hirschmann (1954) signalant la présence de *R. oryzae* au voisinage de racines de *Caltha palustris*; les individus trouvés étaient d'ailleurs beaucoup plus grands que le type; le dernier auteur suggérait également la synonymie possible de cette espèce avec *Tylenchorhynchus gracilis* (De Man 1880), Filipjev 1936. Nous pensions à l'époque que les individus trouvés par nous sur le riz au Cameroun,

1) Accepté: le 29 janvier, 1957.

se rapportaient à une variété de grande taille de *Radopholus oryzae*, synonyme de *Radopholus gigas* et peut-être de *T. gracilis*.

Deux articles ultérieurs de Hirschmann (1955 a et b) montraient que *Tylenchorhynchus gracilis* devait être renommé *Radopholus gracilis* (De Man 1880), Hirschmann 1955 et était synonyme de *R. gigas* Andrassy 1954 et *R. oryzae* (v. Breda de Haan, 1902), Thorne 1949. La synonymisation avec cette dernière espèce demanderait l'appui d'infections positives de *R. gracilis* typique sur le riz.

En reprenant les échantillons du *Radopholus* trouvé sur riz au Cameroun il se révéla qu'il s'agissait en fait d'une espèce présentant suffisamment de caractères originaux pour être distinguée de *R. gracilis* (= *R. oryzae*) et considérée comme nouvelle.

Radopholus lavabri n.sp.

Dimensions:

♀: L = 2,203-3,313 mm — a = 39,9-48,9 b = 5,5 c = 16,4-25,9 V = 52,0-55,1 % — stylet = 42-45,5 μ

♂: L = 2,109-2,353 — a = 43,9-46,5 — b = 4,3 — c = 18,3-19,6 T = 34,2 % stylet = 38-42 μ spicules = 43 μ — gubernaculum = 14-15 μ.

Description:

Femelle:

Cuticule avec stries transversales distinctes, larges de 2-2,5 μ; champ latéral occupant un peu moins du $^1/_3$ de la largeur correspondante, constitué par 4 lignes longitudinales crénelées et traversées par chaque striation transversale; le champ latéral débute à peu près au niveau de la base du stylet, les deux lignes centrales se rejoignant un peu postérieurement à l'anus, les deux lignes latérales s'évanouissant un peu plus loin; à partir du premier $^1/_3$ de la queue le champ latéral n'est plus marqué que par une ligne centrale, mince, sinueuse, de moins en moins nette vers l'extrémité et surtout visible par les discontinuités des stries transverses. Région labiale hémisphérique, portant 4 à 5 stries (5 à 6 annules); lèvres soudées, sans incisure les séparant du corps; largeur labiale: 17-18 μ. Armature céphalique très développé. Stylet de 42 à 45,5 μ; protorhabdion légèrement plus long que le cheilorhabdion; boutons basaux arrondis, sans ornementation; junction de la glande oesophagienne à 2 μ en arrière du stylet. Bulbe médian sphérique avec valvules bien développées. Partie glandulaire de l'oesophage recouvrant loin en arrière la partie antérieure de l'intestin.

Anneau nerveux un peu antérieur au pore excréteur. Pore excréteur à 6,2-7 % de la longueur totale. Hemizonide lenticulaire située à 6-8 μ en avant du pore excréteur. Vulve à 52-55,1 %, en fente profonde. Deux ovaires droits de 570-710 μ de long, l'antérieur généralement plus court; ovocytes en une seule file, sauf à la partie proximale; spermathèque présente pour chaque ovaire. Queue conique — allongée, à extrémité obtuse portant généralement une petite pointe effilée de forme et de longueur variables; longueur de la queue = 2,5-3,7 largeurs anales. Phasmides situées à 42-45 μ de l'extrémité postérieure. Dimensions moyennes des oeufs déposés dans les tissus de la plante: 145 × 45 μ.

Mâle:

Caractères généraux identiques à ceux de la femelle. Stylet plus court: 38-42 μ et largeur labiale plus faible: 14,5-15,5 μ. Champ latéral légèrement différent: les 4 lignes longitudinales ne sont pas traversées par toutes les stries transverses mais par une sur 2 ou 3, de façon irrégulière et souvent incomplète (cf. fig. 1, F); le champ latéral s'évanouit au début de la bourse ne subsistant que par la ligne dorso-latérale qui elle-même disparaît à moitié de la queue. Spicules courbes, de 43 μ de long. Gubernaculum droit, simple, de 14-15 μ de long. Testicule unique. Bourse crénelée, enveloppant près des $^4/_5$ de la queue. Queue conique — allongée, présentant le même type d'extrémité que chez la femelle; longueur de la queue = 3,8-4 largeurs anales.

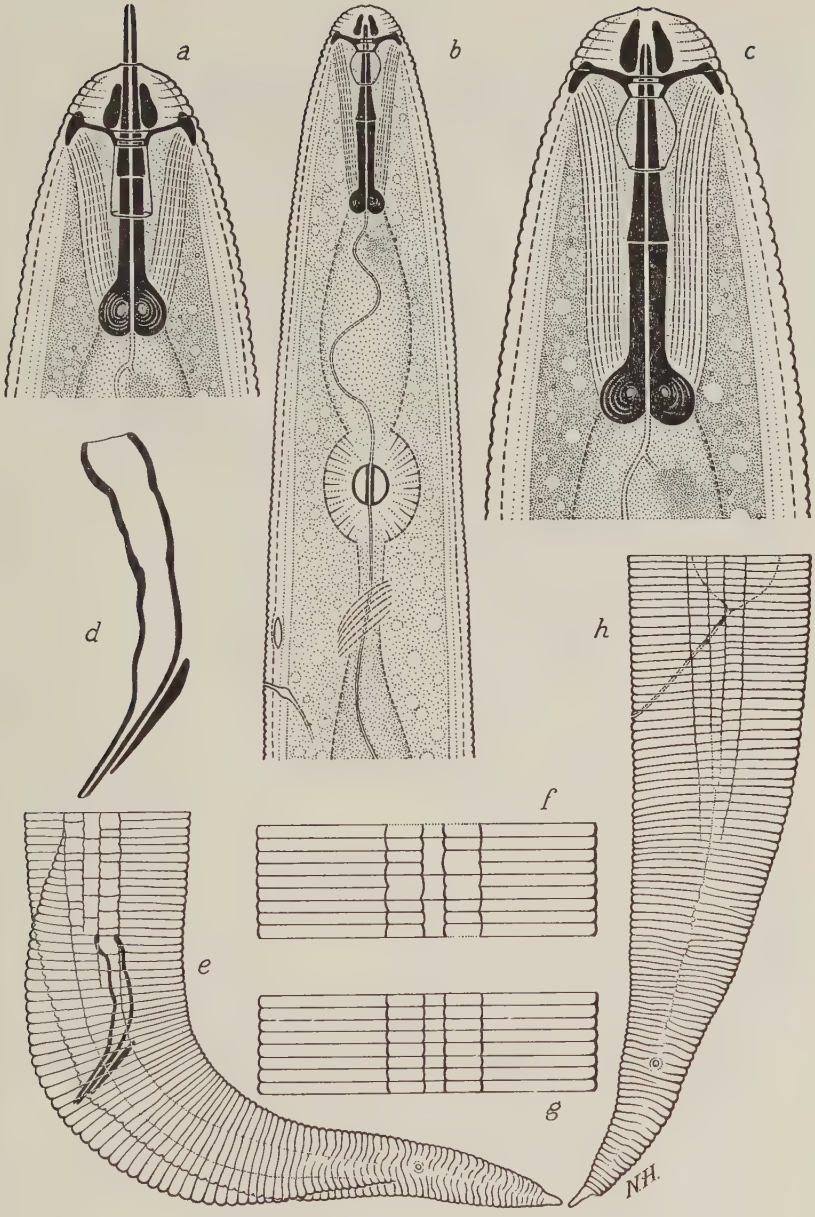
Specimen type: Femelle; tube N° 85, Laboratoire de Nématologie, I.D.E.R.T., Abidjan.

Allotype: Mâle; tube N° 86; Laboratoire de Nématologie, I.D.E.-R.T., Abidjan.

Hôte type: *Oryza* sp.

Localité type: Koko, près de Yagoua, rizière en bordure du Logone (Cameroun Français).

Radopholus lavabri se différencie de *R. gracilis* (De Man 1880), Hirschmann 1955, par sa région labiale nettement hémisphérique, la longueur presque double du stylet, les dimensions supérieures des spicules et du gubernaculum et les caractères du champ latéral: chez aucune espèce du genre *Radopholus* en effet le champ latéral n'est traversé par les stries transverses, tout au plus cela se remarque-t-il à la partie postérieure du corps chez le mâle de *R. gracilis*; enfin la queue de *R. lavabri* est beaucoup moins effilée que celle de *R. gracilis*.



Radopholus lavabri n.sp.-Mâle: A: tête; D: spicules et gubernaculum; E: queue; F: champ latéral. — Femelle: B: partie antérieure; C: Tête; H: queue; G: champ latéral. (Gross. a, c, d: x 1130; b, e, h: x 550; f, g: schématique)

SUMMARY

A description is given of *Radopholus lavabri* n.sp., found on the roots of rice plants in the French Cameroons (West Africa). The species differs from *R. gracilis* (De Man 1880) Hirschmann 1955 in the following: lip region clearly hemispherical, spear nearly twice as long, spicules and gubernaculum larger, tail not so thin. The lateral field is not crossed by transverse lines in any species of the genus *Radopholus* except in the terminal portion of the body of male *R. gracilis*.

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(*Nematologica* II (1957): 149-151)

NOTE ON THE NOMENCLATURE OF THE CEREAL ROOT EELWORM [1])

BY

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The species of *Heterodera* parasitic on cereals has recently been attracting increasing attention both in Europe and elsewhere. Amongst the Heteroderas it takes third place after the sugar beet and potato root eelworms as an important pest of agricultural crops. It is widespread in Europe and occurs also in Canada, Australia and Japan. Surveys and research into the biology and control of this nematode have recently been carried out in Britain, Denmark, Germany and Holland.

In current literature the cereal root eelworm is referred to sometimes as *Heterodera major,* sometimes as *H. avenae.* This disagreement as to its correct scientific name is bound to lead to confusion. Since there now exists, in Nematologica, an international journal for the publication of matters concerning nematodes of agricultural importance, it seems appropriate to re-state the history of the nomenclature of this nematode so that agreement may be reached as to its scientific name.

It will be remembered that until 1940 (Franklin, 1940) the cyst-forming plant-parasitic nematodes were generally regarded as one species, *Heterodera schachtii,* although it was recognized that there were "strains" with different host preferences, e.g. "potato strain", "oat strain", "beet strain". Some of these "strains" had been given the status and name of variety, subspecies or even species, but morphological differences between them had not been clearly defined and most workers regarded them all as belonging to the species *H. schachtii.* The cereal root eelworm was one of those which was early recognized as a distinct biological race and, later, morphological differences were observed between it and the sugar beet eelworm. The history of the changes in name and status which this nematode has undergone as a result of these observations is briefly as follows.

In the Danish publication *Tidsskrift for Landbrugets Planteavl* the oat eelworm ("havreaal") was referred to as *Heterodera schachtii* in the annual "Oversigt over Landbrugsplanternes Sygdomme" for the

*) Received for publication: November 12, 1956.

year 1894 (Rostrup, 1896) and following years. In the same journal Hansen (1904) published a paper on the oat eelworm in which he clearly recognized it as a distinct biologic race as compared with the beet eelworm, but he did not name it as such. He gave a brief account of the life history, and measurements of cysts, eggs and males, but these appear to have been taken from Vaňha & Stoklasa's (1896) description of the sugar beet eelworm. In the "Oversigt over Landbrugsplanternes Sygdomme i 1907" by Mortensen, Rostrup & Kølpin Ravn (1908), the oat eelworm was for the first time referred to as "*H. schachtii* var. *avenae*". There was no description accompanying this name, merely the record of the occurrence of the nematode. Nor does there appear to be any reference to Hansen's paper of 1904. In later years the varietal name *avenae* is frequently used in the "Oversigt", but there appears never to have been a description of the nematode nor a reference to a description of it published elsewhere.

The first worker to record definite morphological differences between the sugar beet and the oat eelworms was Schmidt (1930). He made measurements of the second stage larvae of the two "strains" and showed that "oat strain" larvae were longer than those of the "beet strain". He therefore named the oat eelworm *H. schachtii* subsp. *major* and the beet eelworm *H. schachtii* subsp. *minor*. This author did not mention the Danish literature on the subject.

Four years later, in his work on nematodes harmful and beneficial to agriculture, Filipjev (1934) described the oat root eelworm and raised it to full specific rank, giving it the name *H. avenae*. He mentioned the Danish publications and Schmidt's work but considered that since the name *avenae* antedates *major* the former was the correct one to adopt.

No one will dispute that in deciding the correct name of an organism, in cases where more than one name has been used, the principles laid down in the International Rules for Zoological Nomenclature should be followed. In this case the relevant principle is laid down in Article 25 which states the Law of Priority as follows (Schenk & McMasters, 1948):

"The valid name of a genus or species can be only that name under which it was first designated on the condition:

a) That (prior to January 1, 1931) this name was published and accompanied by an indication, or a definition, or a description; and

b) That the author has applied the principles of binary nomenclature.

Opinion 1. The word "indication", above, is to be construed as follows: A. With regard to *specific* names, an "indication" is (1) a bibliographic reference, or (2) a published figure (illustration), or (3) a definite citation of an earlier name for which a new name is proposed."

At the meeting of the International Commission on Zoological Nomenclature held in Paris in July 1948 (HEMMING, 1950) the Commission agreed to recommend

"that words should be inserted in the *Règles* to make it clear that the citation of the name of the host species of a parasitic species, unaccompanied by any other particulars does not constitute an "indication" of the purpose of Article 25 ..."

Following the principles thus laid down it seems clear that in the case of the cereal root eelworm the name *major,* although given later than *avenae,* is the correct specific name because, so far as the writer has been able to find out, *avenae* was not published together with an adequate "indication". It should, however, be emphasised that the important point is not so much *which* name should be adopted, but that only *one* should be used, and that that one should be chosen in accordance with the International Rules of Zoological Nomenclature. Unless, therefore, some relevant publication or reference has been overlooked, it is clear that the correct name for the cereal root eelworm is *Heterodera major* (O. SCHMIDT, 1930) FRANKLIN, 1940.

ZUSAMMENFASSUNG

In der nematologischen Literatur wird der Hafernematode zuweilen *Heterodera major* und zuweilen *Heterodera avenae* genannt. Um den richtigen Namen zu entscheiden sind die Geschichte der Nomenklatur des Nematoden sowie die Regeln der Internationalen Kommission für Zoologische Nomenklatur zur Feststellung herangezogen worden. Es wird gefolgert, dass der richtige Name *Heterodera major* (O. SCHMIDT, 1930) FRANKLIN, 1940 ist.

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(*Nematologica* II (1957): 152-158)

OBSERVATIONS ON *ECPHYADOPHORA TENUISSIMA* DE MAN, 1921.*†

BY

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Among rare and incompletely described nematode species regarded as taxonomic curiosities is *Ecphyadophora tenuissima*. De Man (1921) described one male specimen as representing a genus of extremely slender, spear-bearing forms with a nondescript esophagus. The male was significantly different from all other nematodes due to its striking caudal alae and needle-like spicules.

A sample of roots and soil from an orange grove yielded one male specimen which, even under examination at 75 × with a dissecting microscope, showed a decided similarity to De Man's figures. Gallons of soil obtained at various times from the same location failed to yield another specimen of this genus. Additional specimens, however, were obtained from five other locations as detailed below.

Living specimens were killed by gentle heat and fixed for 24 hours in TAF medium (Courtney et al., 1955). All fixed nematodes were stained with acid fuchsin in lactophenol, and gradually transferred to glycerin using the technique proposed by Baker (1953). This treatment rendered the specimens in an excellent state of preservation, in all respects, except for the buccal stylets which became somewhat obscure.

The living worms were slow-moving and frequently assumed positions with their bodies in multiple coils. The stylet was composed of 2 distinct parts, with the posterior portion somewhat longer than the anterior portion. In the living specimens, the esophagus was indistinct and difficult to distinguish from the intestine. In the female the prerectum appeared light while the anus appeared to be situated about three-fifths the distance from the vulva to the tail.

The measurements, habitats, and localities of each of the populations studied are presented below.

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*) Florida Agricultural Experiment Station Journal Series, No. 536.
†) Received for publication: November 17, 1956.

Ecphyadophora tenuissima de Man, 1921

(1) From Haines City, Florida. 1 ♂: L = 673 μ; a = 96.1; b = 3.9; c = 9.3; T = 22 %; stylet 10.3 μ long; stylet knobs 1.8 μ wide; caudal ala length 7 μ, width 2.8 μ; spicule length 14.8 μ, nerve ring 77 μ from anterior end; excretory pore 105 μ from anterior end. Slide 4, Tray 1, Cabinet C-2724, Nematode Collection, Citrus Experiment Station, Lake Alfred, Florida.

(2) From Ruurlo, Holland. 7 ♀: L = 978 μ (933-1, 020 μ); a = 158.9 (133.3-197.4); b= 6.4 (5.6-7.7); c = ?; V = 23(19-27)75 (73-79); stylet 9.7 μ (9.3-10.1 μ) long. 2 ♂: L = 958 μ (935-980 μ); a = 159.4 (155.8-163.0); b = 6.0 (5.5-6.6); c = 12.2 (11.7-12.7); T = 24 (22-26) %; stylet 9.6 μ long. Neotype (♂), slide 6, and 2 ♀, slide 7, Tray 1, Cabinet C-2724, Nematode Collection, Citrus Experiment Station, Lake Alfred, Florida. 3 ♀, 1 ♂ in University of California Collection, Berkeley, and 3 ♀ in author's possession.

(3) From Yeehaw Junction, Florida. 2 ♀; L = 625 μ (536-714 μ); a = 107.4 (95.7-119.0); b = 5.0; c = ?; V = 22(21-24)74; stylet 7.2 μ long; excretory pore 105 μ from anterior end. Two ♀ in author's possession.

(4) From Lake Alfred, Florida. 2 ♀: L = 807 μ (791-822 μ); a = 145.8 (136.4-155.1); b = 3.8; c = 12.1; V = 18(17-20)70 (69-70); stylet 8.0 μ (7.2-8.8 μ) long. 1 ♂: L = 630 μ; a = 105.0; b = ?; c = 9.5; T = 25 %; spicule 15.7 μ long. Two ♀ and 1 ♂ in author's possession.

(5) From Fort Pierce, Florida. 3 ♀: L = 726 μ (659-838 μ); a = 113.3 (109.8-116.4); b = 5.6 (5.0-6.2); c = 12.5 (11.8-13.1); V = 17(16-18)75(74-76); stylet 7.7 μ long; nerve ring 76 μ from anterior end; excretory pore 103 μ from anterior end. One ♀, slide 8, Tray 1, Cabinet C-2724, Nematode Collection, Citrus Experiment Station, Lake Alfred, Florida. Two ♀ in author's possession.

(6) From Jacksonville, Florida. 5 ♀: L = 888 μ (852-927 μ); a = 137.9 (129.4-154.5); b = 7.0 (6.5-7.6); c = 10.8 (10.4-11.3); V = 18(16-20)70(69-72); stylet 8.2 μ long; nerve ring 80 μ from anterior end; excretory pore 109 μ from anterior end. 2 ♂: L = 892 μ (843-941 μ); a = 156.4 (150.5-162.2); b = 6.6 (5.7-

7.4); c = 10.3 (10.1-10.5); T = 22 (22-23) %; spear 7.6 μ long; spicules 13.2 μ (12.9-13.5 μ) long; caudal ala 6.3 μ long, width 3.0 μ. One ♀, slide 9, Tray 1, Cabinet C-2724, Nematode Collection, Citrus Experiment Station, Lake Alfred, Florida. Six ♀ and 2 ♂ in author's possession.

Description Emended:

Body extremely slender, without apparent annulations, lateral fields, papillae, or setae, usually assuming a straightened position when killed by heat. Lip region bluntly rounded in shape with a slight depression at oral opening when viewed in optical longitudinal section (Fig. 1, C), continuous with body contour. Lips obscure, completely amalgamated, about 2.4 μ in length, usually assuming a lighter coloration than rest of body in preserved specimen. Stylet from 7.2-10.3 μ long, bearing rounded knobs measuring about 1.8 μ in width. Esophagus mostly obscure, encircled by nerve ring approximately midway in its length about 76-84 μ from anterior end. Metacorpus not observed, isthmus broadly crenate in outline, posterior bulb somewhat expanded, elongate, sometimes terminating in a broadly digitate extension (Fig. 1, B). Neither esophageal glands nor gland nuclei observed. Excretory pore distinct with walls of excretory canal weakly sclerotized; situated about 103-115 μ from anterior end of worm. Intestine somewhat indistinct.

Female gonad single and prodelphic, containing single row of nucleated oöcytes. Spermatheca distinct, not set-off from uterus, often containing numerous sperm. Vulva very conspicuous, always located at point where body abruptly narrows (Fig. 1, J).

Testis single, distinct, containing single row of nucleated cells. Seminal vesicle containing numerous oval to angular shape sperm, mainly 2 and 3-ranked (Fig. 1, A). Spicules needle-like, curved slightly, with or without apparent cephalation, 14.6 μ (13.2-15.7 μ) long (Fig. 1, E and L). Gubernaculum not observed. Caudal alae flap-like, without striations, of varying dentoid-cylindrical shape (Fig. 1, F, M-N). Distinct crenation of cuticle on dorsal side of body visible in spicule area. Body narrowing abruptly to form cylindrical tail which gradually tapers beginning at about 3/5ths of its total length from the anterior end.

Terminus rounded, varying from sub-digitate (Fig. 2, C) to hemispherical (Fig. 1, O-S) shape.

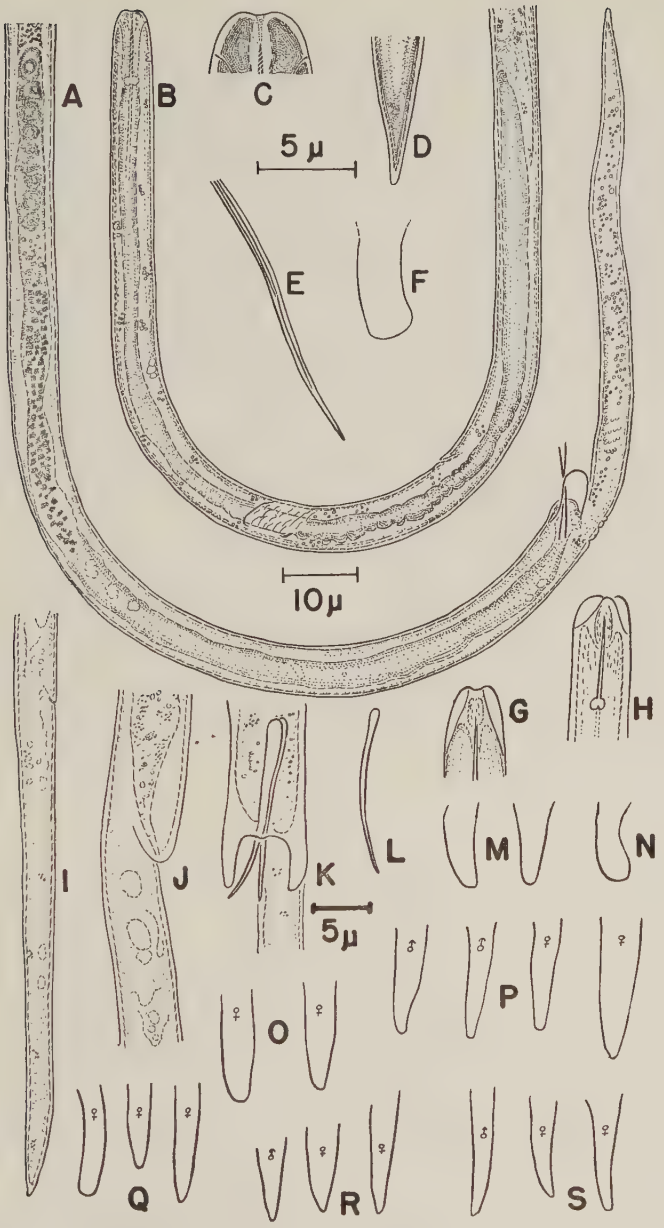

Fig. 1 — *Ecphyadophora tenuissima* de Man. A-F. Male from citrus. A. Reproductive organs and tail. B. Esophageal region. C. Lip region. D. Tail terminus. E. Spicule. F. Caudal ala. G. Lip region, male from sugar cane. H. Lip region, female from sugar cane. I. Tail, *Juniperus* specimen. J. Vulva, *Juniperus* specimen. K. Ventral view of spicules and caudal alae, sugar cane specimen. L. Spicule, *Juniperus* specimen. M-N. Caudal alae. M. *Juniperus* specimens. N. Dutch specimen. O-S. Tail termini. O. Meadow soil specimens. P. Dutch specimens. Q. Tomato soil specimens. R. Sugar cane specimens. S. *Juniperus* specimens.

Habitats and Localities:

One male obtained at a 5 ft. depth from soil and roots of Valencia orange on rough lemon (*Citrus limon* (L.) Burman) rootstock, Jack Barry Grove, Highway Number 27, 2 miles north of Haines City, Florida; collected by A. C. TARJAN on January 21, 1956. Two males and 7 females from marshy waste land near Ruurlo, Holland; collected by J. W. SEINHORST in December, 1952 and kindly sent to the author by M. W. ALLEN on May 10, 1956. Two females from roots and soil of pasture grass taken at a 5 inch depth along the roadside of Route 60, about 2 miles northwest of Yeehaw Junction, Florida; collected by A. C. TARJAN on May 14, 1956. One male and 2 females from sugar cane roots (*Saccharum officinarum* L.) and soil at a 10 inch depth, southwest corner by railroad tracks, Citrus Experiment Station, Lake Alfred, Florida; collected by A. C. TARJAN on May 28, 1956. Three females from tomato (*Lycopersicon esculentum* L.) soil, Immokalee fine sand, Indian River Field Laboratory, Fort Pierce, Florida; collected by A. E. KRETSCHMER in March, 1956 and kindly sent to the author by W. H. THAMES during the same month. Two males and 5 females from *Juniperus silicicola* (Small) Bailey roots and soil, Grant Haven Estate, Jacksonville, Florida; collected by B. G. CHITWOOD on March 20, 1956 and kindly sent to the author by C. I. HANNON.

Comparisons:

The description by DE MAN (1921) of a single male specimen gives the length of the worm as 0.9 mm while a = 150, b = 5.3, and c = 10.7. His drawings show the head to be perfectly rounded and the stylet comprised of 2 parts, the basal portion bearing knobs (Fig. 2, A). The cephalated spicules are associated with distinctive caudal alae which envelop and overlap the beginning of the tail (Fig. 2, B). When viewed laterally, the posterior portions of these alae are ventrally convex-convoid in shape (Fig. 2, D). The terminus of the tail is figured as bearing a mucor-like projection (Fig. 2, C).

The single male obtained from rough lemon roots and soil and illustrated in Fig. 1, A-F differed most from DE MAN's specimen in its size, alpha measurement, spicule and caudal ala shape, and terminus shape. Also it bore a distinct crenation of the cuticle on the dorsal body surface in the spicule area which likewise was apparent on the other males examined. Whereas in all specimens observed, a

distinct nerve ring and excretory pore was apparent, the anus and rectum on several females was obscure and indeterminate.

The average size of specimens from each of the populations obtained varied considerably. Those from sugar cane, *Juniperus,* and Dutch marshy wasteland averaged over 200 μ longer than the average length of nemas from tomato, meadow and citrus soils. Also some of the latter group exhibited tails with hemispherical termini (Fig. 1, O and Q) whereas specimens in the former group tended to have tails with minutely rounded termini (Fig. 1, P. R. and S). However, it is felt that these differences as well as those between populations in a, b, and c measurements, represent only geographical variations and are not of sufficient magnitude to be considered as specific differences.

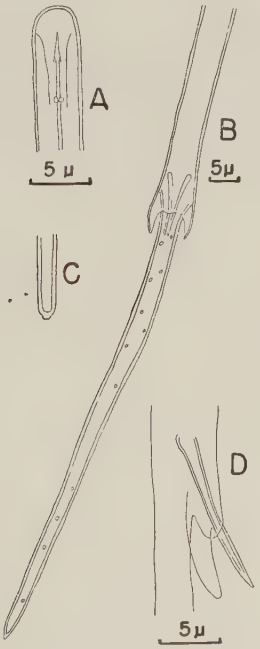

Fig. 2 — *Ecphyadophora tenuissima* de Man. A. Cephalic region. B. Posterior region of male, ventral view. C. Tail extremity. D. Anal region profile (after De Man).

Thorne (1949) considered *Ecphyadophora* as a genus of uncertain position within the family *Tylenchidae* on the basis of the limited information contained in De Man's description of a single male. The present study, which has included 2 larval specimens, confirms the placement of the genus in the order *Tylenchida,* in view of the presence

of caudal alae and the origin of the spear as a true stomatostyle. Members of the *Tylenchidae* usually exhibit cuticular annulation and always an esophageal metacorpus. The absence of these features in *Ecphyadophora* necessitate the provisional transfer of the genus from the *Tylenchidae* to the *Neotylenchidae*.

SUMMARY

Nineteen females and five males of *Ecphyadophora tenuissima* de Man 1921 from five locations in Florida and from Ruurlo, Holland have been studied. Geographical variations have been noted and an emended description of the species presented. A male from the Dutch population is designated as neotype.

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(*Nematologica* II (1957) : 159-174)

VARIATION WITHIN THE NEMATODE GENUS *PRATYLENCHUS*, WITH THE DESCRIPTIONS OF *P. HEXINCISUS*, N. SP. AND *P. SUBPENETRANS*, N. SP. [1] [2]

BY

D. P. TAYLOR [3] AND W. R. JENKINS [4]

The genus *Pratylenchus* Filipjev, 1934, consists of 12 described species and two varieties, all of which are obligate endoparasites of plants. Members of this genus are considered to be among the most serious of root parasitic nematodes.

Considerable confusion existed regarding the species included within this genus until 1953 when *Pratylenchus* was revised by SHER and ALLEN. At that time the following ten species were recognized: *P. pratensis* (de Man, 1880) Filipjev, 1936; *P. coffeae* (Zimmermann, 1898) Goodey, 1951; *P. scribneri* Steiner, 1943; *P. brachyurus* (Godfrey, 1929) Goodey, 1951; *P. vulnus* Allen et Jensen, 1951; *P. zeae* Graham, 1951; *P. penetrans* (Cobb, 1917) Sher et Allen, 1953; *P. thornei* Sher et Allen, 1953; *P. goodeyi* Sher et Allen, 1953; and *P. minyus* Sher et Allen, 1953. Unfortunately, their review did not include *P. tumidiceps* Merzheyevskaya, 1951. Since then *P. pratensis* var. *tenuistriatus* Meyl, 1953, *P. pratensis* var. *bicaudatus* Meyl, 1954 and *P. steineri* Lordello, Zamith et Boock, 1954, have been described.

Variation occurring in *Pratylenchus* has not been reported to any extent. As with other species of nematodes, a range of certain values [5] is published with the description of new species of *Pratylenchus*, but in the majority of descriptions neither the means of these values nor

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5) The term "values" used here and afterwards refers to: L = over-all length; a = length divided by greatest width; b = length divided by esophageal length (measured to the junction of the intestine); c = length divided by length of tail; and V = distance of vulva from anterior end expressed as a percentage of the total length.

the number of specimens measured have been given. For example, Sher and Allen (1953) gave only a range for the ten species which they described or redescribed and mentioned neither the number of specimens nor the means of values.

Five variations in the shape of the tail of *P. brachyurus,* ranging from a rounded to a square terminus, found in a naturally occurring population, were illustrated by Godfrey in 1929. However, no mention was made of the relative frequency of these variations nor were any other variations discussed.

Although species identification is based upon morphological characters, it can be greatly facilitated by the use of certain measurements if the range and variation of the measurements is known. Therefore, it was attempted to demonstrate the amount of variation that occurred in natural populations with regard to these values as well as to certain morphological characters.

METHODS

Specimens used in morphological studies came from the following sources: *P. zeae* from field corn four miles northeast of Snow Hill, Worcester County; *P. penetrans* from strawberry from Rayner Brothers' Nursery, Wicomico County; *P. hexincisus,* n. sp., from sweet corn from Le Cates farm, Kent County; and *P. subpenetrans,* n. sp., from pasture grasses on the A. H. Smith farm, Upper Marlboro, Prince Georges County, all in Maryland.

Cultures of *Pratylenchus* species were obtained by planting seeds of the crop from which the original sample was taken into the naturally infested soil. These cultures were maintained for several months to allow the nematode populations to build up, at which time the soil and roots were processed for the recovery of *Pratylenchus* specimens. Adults were placed on slides in three per cent formaldehyde. By means of a Zeiss Drawing Apparatus drawings of each specimen were made from which measurements were taken.

DESCRIPTIONS OF NEW SPECIES

Pratylenchus hexincisus, new species (Figure 1)

VALUES:

95 Females: L = .436 ± .044 mm. (.342-.540 mm.) [1]); a = 22.6 ±

1) The first figure for each dimension is the mean of the population while the second figure represents the standard deviation from the mean. The observed range is included in parentheses.

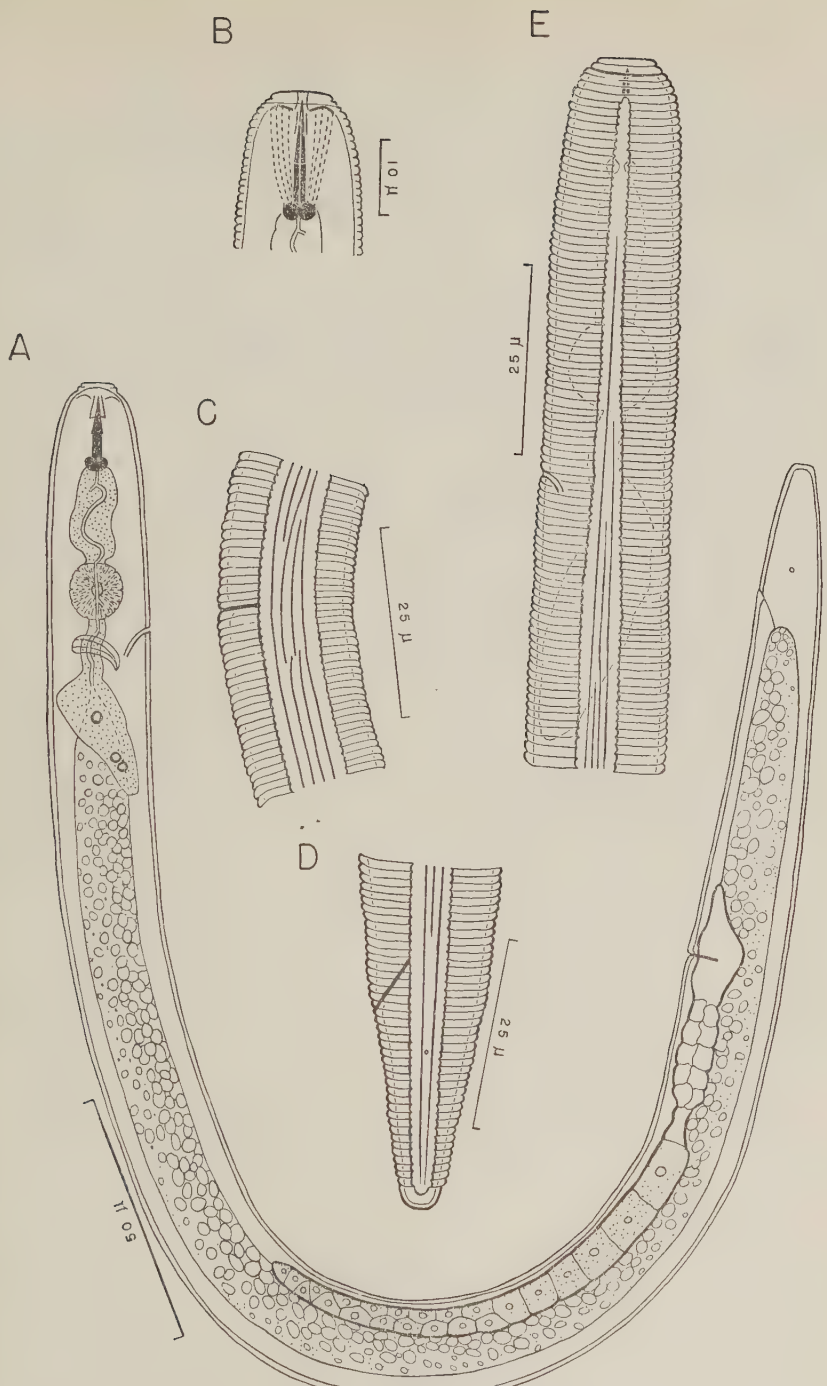

Figure 1. — *Pratylenchus hexincisus* n. sp. A. Adult female. B. Head of female. C. Lateral field and cuticular striations in vulvar region. D. Lateral field and cuticular striations on posterior portion. E. Lateral field and cuticular striations on anterior portion.

2.5 (18.2-28.8); b = 7.2 ± 0.6 (5.9-8.4); c = 18.6 ± 1.3 (16.1-22.7); V = 78.0 % ± 1.9 % (75.3-82.2 %); Stylet = 15 μ (14.5-15.4 μ).

DESCRIPTION:

Female: The short and somewhat stout body tapers towards both ends. The cuticle is marked with transverse striations about one micron apart, occurring slightly farther apart on the tail of some specimens. The lateral field occupies about one-third of the body diameter in the vulvar region, originates as a narrow groove at the base of the stylet guiding apparatus, and contains six incisures throughout most of its length. Outer incisures are crenate while the inner incisures are somewhat irregular, particularly in the vulvar region where breaks and oblique lines frequently occur (Figure 1-C). Only four incisures occur on the tail. No cervical papilla (deirid) was observed. Phasmids occur in the anterior $^1/_4$-$^2/_5$ of the tail. The unstriated tail-tip is rounded.

The low, flattened lip region bears one striation and its lateral margins are rounded in outline. Lateral margins of the sclerotized cephalic framework extend posteriorly about one micron into the body cavity. The stylet guiding apparatus is 3.2-3.6 μ long. The short, stout stylet has spherical basal knobs.

The dorsal esophageal gland duct opens into the slightly convoluted lumen of the esophagus 2.2 μ (1.7-2.5 μ) posterior to the stylet base. The esophagus consists in part of the distinct corpus and an ovoid middle bulb which contains a heavily sclerotized valvular apparatus. The excretory pore is located in the region of the isthmus; the nerve ring surrounds this same structure. Esophageal glands ventrally overlap the intestine, which contains granules that are larger and more dense towards the ventral surface. The dorsal esophageal gland nucleus is larger than the subventral nuclei. The intestine terminates in an oblique rectum that opens through the cuticle by means of a conspicuous anus.

The single outstretched ovary extends anteriorly about one-half of the body length. Except for a double row in the anterior portion, the ovary contains a single row of oocytes. An oviduct connects the ovary and the cellular uterus. The cuticularized vagina extends transversely about one-half the body diameter and opens by means of a prominent vulva. The short post-uterine branch is about equal to the body diameter at the vulva. No spermatheca was observed.

Male: Unknown.

TYPE HOST: Roots of corn, *Zea mays* L. TYPE LOCALITY: Le Cates farm, Kent County, Maryland. TYPE SPECIMENS: University of Maryland, College Park, Maryland.

DIAGNOSIS: *P. hexincisus,* n. sp. can be distinguished from all other described *Pratylenchus* species by the presence of six incisures in the lateral field throughout most of its length.

In addition it can be distinguished from *P. pratensis, P. pratensis* var. *tenuistriatus, P. thornei, P. zeae, P. goodeyi, P. vulnus, P. penetrans,* and *P. subpenetrans* by the presence of one striation (two annulations) on its lip region. *P. hexincisus* differs from *P. brachyurus* by the shorter stylet, more anterior position of the vulva, and the non-angular lip margins of the former species. From *P. coffeae* it can be distinguished by the absence of males, its shorter and stouter body, and its shorter post-uterine branch. The shorter stylet and more anterior vulva position of *P. hexincisus* distinguishes it from *P. steineri. P. hexincisus* can be distinguished from *P. tumidiceps* by its gradual reduction in body diameter both anteriorly and posteriorly, its shorter esophagus, and by the lack of males. *P. hexincisus* most closely resembles *P. scribneri* and *P. minyus.* In addition to having six incisures, it differs from the former by its smaller size and longer lateral field and from the latter by the more anterior position of the vulva.

Pratylenchus subpenetrans, new species (Figure 2)

VALUES:

100 Females: L = .400 ± .044 mm (.330-.481 mm.); a = 24.2 ± 2.0 (18.4-27.7); b = 6.0 ± 0.4 (5.0-7.2); c = 18.2 ± 1.1 (16.2-21.4); V = 80.1 ± 1.1 % (77.4-82.9 %); Stylet = 15.8 μ (14.9-16.5 μ).

100 Males: L = .390 ± .030 mm. (.329-.472 mm.); a = 27.7 ± 2.0 (23.7-32.1); b = 6.1 ± 0.5 (5.0-7.4); c = 17.9 ± 1.2 (14.7-21.0); Stylet = 14.6 μ (14.3-15.2 μ).

DESCRIPTION:

Female: The short, somewhat slender body tapers anteriorly and posteriorly. Its cuticle is marked with transverse striations about one micron apart. The lateral field originates as a narrow groove at the base of the stylet guiding piece and occupies about one-third of the body diameter in the vulvar region. Incisures originate in the esophageal region so that four are present at the junction of the esophagus and the intestine. These continue longitudinally for the entire body

length until they disappear on the tail. The outer two incisures are crenate while the inner incisures are straight with no irregularities. No cervical papilla was observed. Phasmids occur about one-half the tail length posterior to the anus. The unstriated tail tip is rounded.

The low, flat lip region is only slightly set off from the body contour

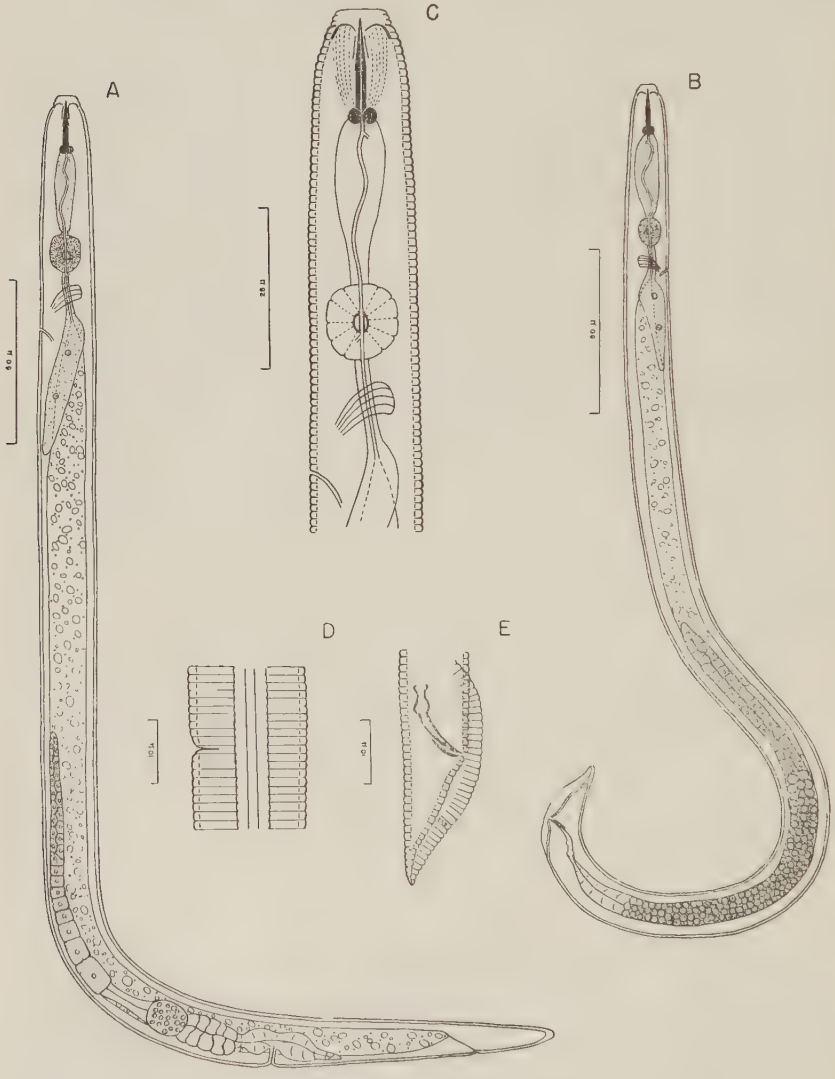

Figure 2. — *Pratylenchus subpenetrans* n. sp. A. Adult female. B. Adult male. C. Anterior end of female. D. Lateral field and cuticular striations in vulvar region. E. Posterior portion of male.

and bears two striations. The lateral margins of the lips are rounded. The lateral margins of the sclerotized cephalic framework extend posteriorly about two microns into the body cavity and are more heavily sclerotized than the rest of this structure. The stylet is short (14.9-16.5 μ) and stout with spherical basal knobs. The guiding apparatus is 3.3-3.8 μ long.

The dorsal esophageal gland duct opens into the convoluted lumen of the esophagus 2.0 μ (1.7-2.2 μ) posterior to the base of the stylet. The anterior portion of the distinct esophageal corpus is more broad than the posterior portion. The ovoid middle bulb contains a heavily sclerotized valvular apparatus. The sub-ventral esophageal gland duct opens into the esophageal lumen immediately posterior to the valvular structure. The nerve ring encircles the narrow isthmus about midway between the middle bulb and the junction of the intestine. The esophageal glands which overlap the intestine are flattened ventrally, appearing long and narrow. The dorsal esophageal gland nucleus is slightly larger than the subventral nuclei. The excretory pore opens through the cuticle opposite or slightly posterior to the junction of the esophagus and the intestine.

Intestinal cells are filled with refractive granules which are about equal in size and evenly distributed. The intestine terminates in an oblique rectum which opens by means of a faint anus.

The single, out-stretched ovary extends anteriorly about halfway to the esophagus. A single row of oocytes is formed in the ovary except for a double row in the anterior portion in the region of multiplication. The oviduct opens into a prominent spermatheca which in turn is followed by a cellular uterus. The vagina extends transversely about one-half the body diameter and opens by means of a conspicuous vulva, the lips of which do not protrude. The post-uterine branch is about one and one-half times longer than the body diameter at that point. The posterior portion of this structure is cellular.

Male: Males occur as frequently as females and although smaller and more slender are morphologically similar, particularly regarding details of the alimentary tract. However, the stylet is shorter, 14.6 μ (14.3-15.2 μ) and the smaller middle bulb tends to be flattened somewhat anteriorly.

The length of the single, out-stretched male reproductive organ is 45 to 50 per cent of the body length. The testis comprises the anterior one-third of the system and contains a multiple row of spermatocytes. The vas deferens and seminal vesicle contain hundreds of small,

spherical refractive sperm. The paired spicules are 14 μ long. They are arcuate dorsally with two swellings, one anteriorly terminal and the other subterminal. The gubernaculum is 5 μ long. The bursa encloses the tail tip and extends slightly beyond the anterior ends of the spicules. Phasmids are located slightly posterior to the middle of the tail.

TYPE HOST: Roots of pasture grasses under natural conditions. Roots of tall fescue, *Festuca elatior* L. under experimental conditions. TYPE LOCALITY: A. H. Smith farm, Upper Marlboro, Prince Georges Country, Maryland. TYPE SPECIMENS: University of Maryland, College Park, Maryland.

DIAGNOSIS: *P. subpenetrans* can be recognized by its small size, the peculiar form of the lateral margins of the cephalic framework, the abundance of males, and by the shape of the spicules.

P. subpenetrans can also be distinguished from *P. brachyurus, P. coffeae, P. scribneri, P. minyus, P. steineri,* and *P. hexincisus* by the presence of 2 striations (3 annulations) on the lip region. From *P. pratensis* it can be distinguished by the absence of striations around the tail terminus and by the presence of a spermatheca. *P. subpenetrans* differs from *P. pratensis* var. *tenuistriatus* by the absence of striations around the tail tip, the presence of a spermatheca, the conspicuous cuticular striations, and by its longer stylet. The cephalic sclerotization of *P. subpenetrans* resembles that of *P. thornei,* but it differs from that species by its smaller size, its shorter stylet, its rounded but not bluntly rounded tail, the presence of a spermatheca, and the more posterior position of the vulva. From *P. zeae, P. subpenetrans* can be distinguished by the presence of males, its smaller size, and the more posterior position of the vulva. The presence of only two lip striations, its much smaller size, and the more posterior position of the vulva separate *P. subpenetrans* from *P. goodeyi. P. subpenetrans* can be distinguished from *P. vulnus* by its smaller size. *P. subpenetrans* differs from *P. tumidiceps* by its tapering body form, the more posterior position of the vulva, and by the abundance of males. *P. subpenetrans* most closely resembles *P. penetrans,* but it can be distinguished from this species by the peculiar cephalic sclerotization, the longer post-uterine branch, the presence of vestigial ovary or uterine tissue posterior to the vulva, and its smaller size. Only about one-tenth of the females of each species were included within the range of L values of the other species. The L values of male specimens had a larger amount of overlapping.

MEASUREMENTS AND VARIATION

L, V, a, b, and c values of four *Pratylenchus* species found in Maryland are given in Table I. Both males and females of *P. penetrans* and *P. subpenetrans* are listed; males of *P. zeae* and *P. hexincisus* are unknown.

Table I

Variations in values of four *Pratylenchus* species occurring in Maryland

Species of *Pratylenchus*	L (microns)	V (%)	a	b	c
P. penetrans					
200 Females					
Mean	527	80.7	25.4	6.4	21.1
Standard Deviation	±43	±1.3	±2.5	±0.8	±2.0
Range	428-622	77.0-84.7	17.3-30.6	4.6-8.1	16.1-27.7
100 Males					
Mean	486		29.4	5.4	18.6
Standard Deviation	±35		±2.6	±0.7	±1.8
Range	386-565		23.9-34.8	3.7-6.9	15.0-25.7
P. zeae					
90 Females					
Mean	521	70.5	21.1	6.3	17.3
Standard Deviation	±51	±2.1	±1.8	±0.8	±2.7
Range	413-639	64.7-74.9	17.0-25.4	5.0-9.6	11.2-24.1
P. hexincisus					
95 Females					
Mean	436	78.0	22.6	7.2	18.6
Standard Deviation	±44	±1.9	±2.5	±0.6	±1.3
Range	342-540	75.3-82.8	18.2-28.8	5.9-8.4	16.1-22.7
P. subpenetrans					
100 Females					
Mean	400	80.1	24.2	6.0	18.2
Standard Deviation	±28	±1.1	±2.0	±0.4	±1.1
Range	330-481	77.4-82.9	18.4-27.7	5.0-7.2	16.2-21.4
100 Males					
Mean	390		27.2	6.1	17.9
Standard Deviation	±30		±2.0	±0.5	±1.2
Range	329-472		23.7-32.1	5.0-7.4	14.7-21.0

When the standard deviation, s, was added to or subtracted from the mean, $\bar{x}$, two-thirds of the populations fell between $\bar{x} + s$ and $\bar{x} - s$. Because the populations studied had this relationship and because individuals within each group agreed with the description of that species, each population consisted of only a single species. Therefore, the variation recorded represents normal variation of single-species populations rather than that of populations of mixed species.

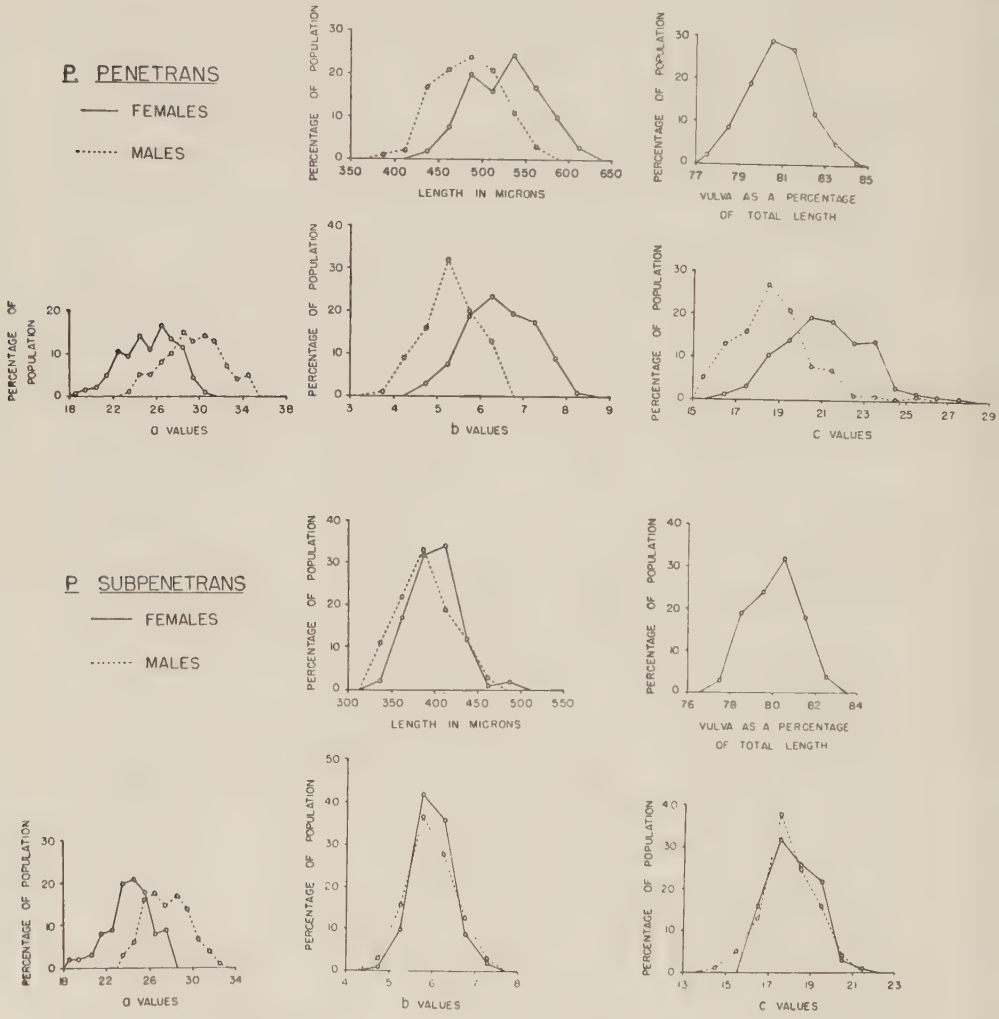

Figure 3. — Distribution of L. V, a, b, and c values of 200 females and 100 males of *Pratylenchus penetrans* an 100 females and 100 males of *Pratylenchus subpenetrans*.

In most cases the standard deviation equaled about one-tenth of the mean. However, the deviations from the means of V were much lower; only 1.0, 3.0, 2.4, and 1.3 per cent of the mean for *P. penetrans, P. zeae, P. hexincisus,* and *P. subpenetrans,* respectively. Because of its low amount of variation, V is regarded as a good character in species differentiation, and its use in diagnostic keys of this genus is acceptable.

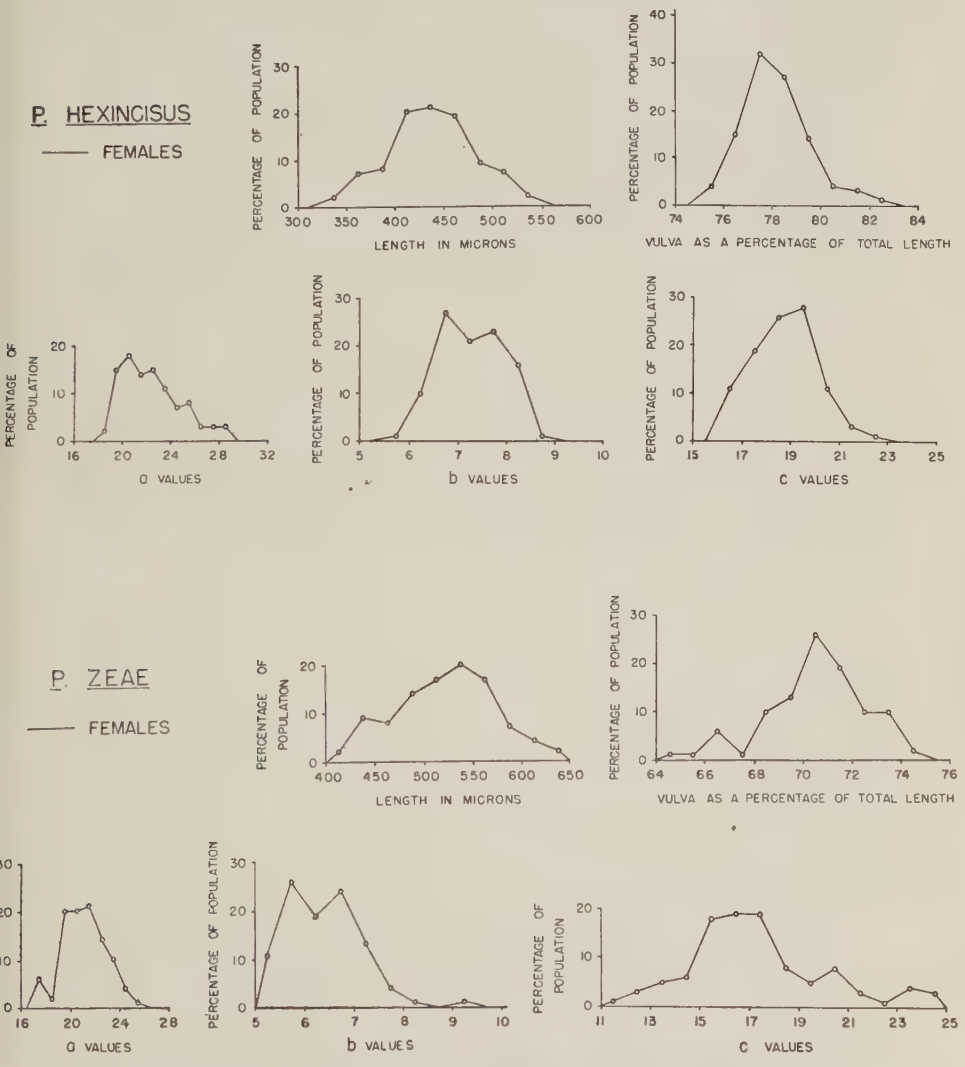

Figure 4. — Distribution of L, V, a, b, and c values of 95 females of *Pratylenchus hexincisus* and 90 females of *Pratylenchus zeae.*

When only the range of each measurement or proportion is published in the description of a species, an erroneous representation of the population might be given. For example, the range of c for *P. penetrans* males was from 15.0 to 25.7. But, by computing $\bar{x} + s$ and $\bar{x} - s$, it was shown that two-thirds of the population was included between 16.8 and 20.4. The minimum observed was only 1.8 units less than $\bar{x} - s$, whereas the maximum observed was 5.3 units greater than $\bar{x} + s$. Therefore, one specimen with a high c (a relatively short tail) increased the upper limit of the range considerable farther from the mean than did the specimen with the lowest c. Publishing only the range of a value may not give an accurate picture of the variation found within a species.

Species identification depends to some extent upon size differences. So that measurements may present a more accurate picture of the population described, it is suggested that the mean and the standard deviation from the mean of values be included with the range in the description of new species.

The distribution of L, V, a, b, and c for 200 females and 100 males of *P. penetrans,* 100 females and 100 males of *P. subpenetrans,* 90 females of *P. zeae,* and 95 females of *P. hexincisus* are illustrated graphically in Figures 3 and 4. In each case the percentage of the population is plotted on the ordinate and the value is plotted on the abscissa. The graphs represent typical distributions for the values of each species, giving additional evidence that the populations studied were composed of single species.

These data demonstrate that wide variation from the mean is normal and that extremes may differ greatly from the reported mean. In no case did as much as half the population occur at the median value. On the other hand, less than one-twentieth of any population occurred at the two extremes. In *P. zeae,* the most variable species in length, the upper extreme was 22.6 per cent greater than the mean while the lower extreme was 20.7 per cent smaller than the mean. The other species varied from a maximum of 23.9 per cent greater to a maximum of 25 per cent smaller.

Shape of the tail-tip was the most variable morphological feature encountered in the four species considered. Figure 5-A-H illustrates tail-tip variations of *P. zeae,* the species most variable for this character. The typical, conical-shape shown in Figures 5-A and 5-B occurred in slightly more than one-half of the specimens. A slight deviation from the typical conical shape, in which the cuticular stria-

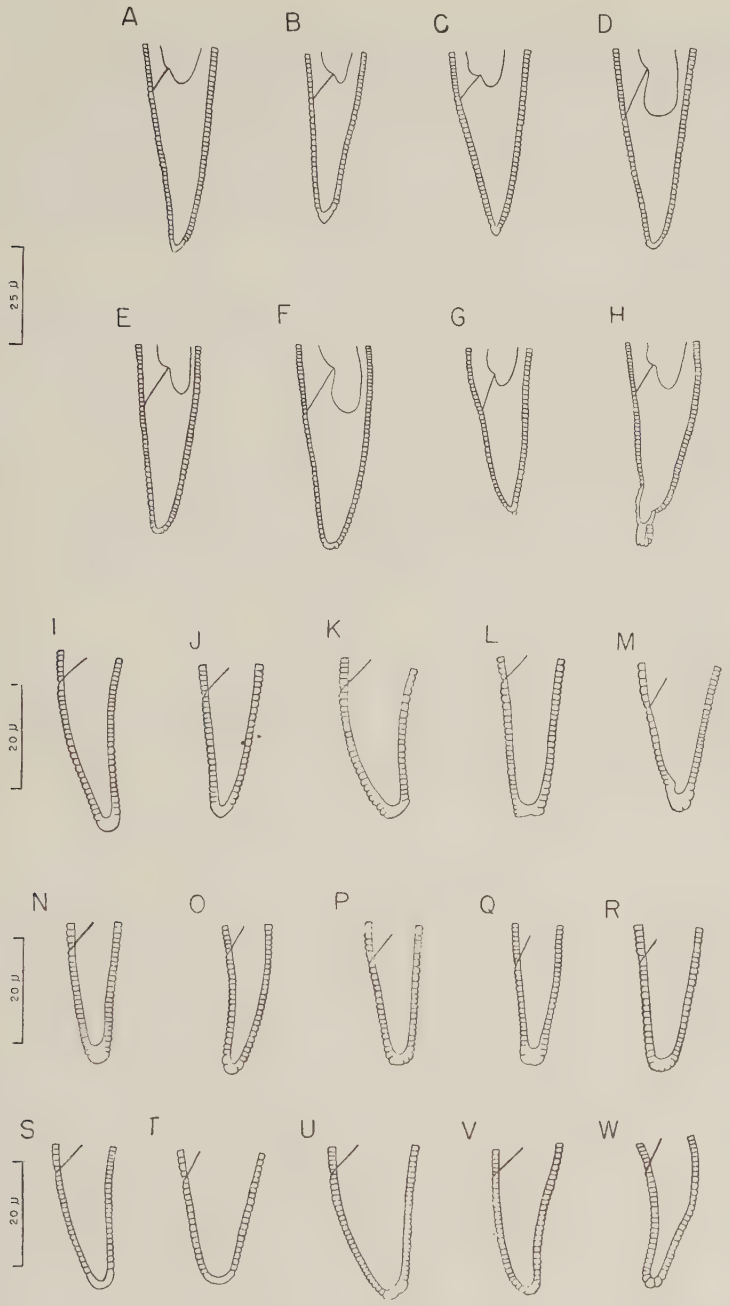

Figure 5. — A.-H. Tail-tip variations in *Pratylenchus zeae*; I-M, *Pratylenchus penetrans*; N-W, *Pratylenchus hexincisus*.

tions are closer to the tail-tip, is shown in Figure 5-C. Figure 5-D illustrates the rather frequently encountered rounded tail-tip. Minor deviations from the rounded form are illustrated in Figures 5-E and 5-F, both of which have striations closer to the terminus. Two aberrant forms found only one time each are shown in Figures 5-G and 5-H. The former has a peculiar annulation on the tail-tip which gives it a definite pointed appearance, while the latter is completely malformed.

Whereas the typical shape occurred in only a little more than one-half of *P. zeae* specimens, female tail-tips of *P. penetrans, P. subpenetrans,* and *P. hexincisus* were far less variable. No major variations in this feature were encountered in males of *P. penetrans* and *P. subpenetrans.* In Figures 5-I, 5-N, and 5-S are illustrated the typical female tail-tips of *P. penetrans, P. subpenetrans,* and *P. hexincisus,* respectively. For each species the typical terminus occurred on at least three-fourths of the specimens examined. Tail-tips of these species are more consistant with regard to shape than those of *P. zeae.* The c value for the latter species was also highly variable (Table I).

Some specimens of *P. zeae* presented the appearance of having striations around the tail-tip (Figures 5-E, 5-F, and 5-G). In Figure 5, specimens of *P. penetrans, P. subpenetrans,* and *P. hexincisus* are shown in which this tendency also existed. Presence of striations around the tail terminus is a species characteristic of *P. pratensis,* and Sher and Allen (1953) used this feature as a diagnostic character of this species. It is possible that specimens of the four species observed, which appear to have or actually do have tail-tip striations, could be identified as *P. pratensis* if only this one character were considered. Such identification based upon cursory examination would result in an incorrect identification of a specimen; therefore, careful examination of all distinguishing characters of several specimens from a single sample should be made before individuals with striations around the tail-tip are identified as *P. pratensis.* One specimen of *P. hexincisus* (Figure 5-W) had obvious tail-tip striations, yet the presence of six incisures in the lateral field and only one striation on the lip region clearly demonstrated that the specimen was not *P. pratensis.* The occurrence of striations around the tail terminus of species other than *P. pratensis* was reported by Sher and Allen (1953), but no illustration of this variation was made nor was there any mention of the species with which this variation was associated.

A clear illustration of the tail-tip of *P. zeae* has never been published

nor was it mentioned in GRAHAM's (1951) original description of this species. SHER and ALLEN (1953) stated that the tail terminus was "almost pointed." Observations of *P. zeae* from Maryland indicate that about one-half of the specimens in the population had an "almost pointed" or conical tail-tip. However, the tail-tip should be described as being quite variable since many specimens were found with striated and aberrant tail-tips.

A dorsal extension of the intestine posterior to the rectum was discovered in all specimens of *P. zeae* observed. This structural arrangement illustrated in Figure 5 was not mentioned by GRAHAM (1951) or SHER and ALLEN (1953). In specimens of *P. zeae* from South Carolina furnished by Dr. GRAHAM, a post-rectal intestinal extension was also observed. Rather than representing a characteristic of a new species, as was originally thought, this structure is regarded as being previously undescribed in *P. zeae.* This intestinal modification may be another diagnostic feature of this species that may aid in its identification since it has not been previously reported in any other species of *Pratylenchus.*

ZUSAMMENFASSUNG [1])

Pratylenchus hexincisus, der mit Zuckermais in Verbindung gebracht wird und *P. subpenetrans,* der aus Weidegräsern wieder gewonnen wurde, werden als neue Arten beschrieben. Die durchschnittliche und normale Abweichung der Mittelwerte von L, V, a, b, und c wurde für eine grosse Anzahl von Individuen von *P. penetrans, P. subpenetrans, P. zeae* und *P. hexincisus* berechnet. Für jede Messung oder jedes Verhältnis innerhalb einer einzelnen Population wurden mindestens zwei Drittel der Individuen zwischen dem Mittelwerte plus oder minus der normalen Abweichung miteinberechnet. Diese Tatsache und der morphologische Vergleich zeigten, dass jede Population, die studiert wurde, aus einer einzigen Art bestand. Darum muss die oben berichtete Abweichung in der Natur intraspezifisch sein. Man erhielt eine normale Verteilungskurve, wenn jede Messung oder jedes Verhältnis graphisch dargestellt wurde. Die V- Werte schwankten am wenigsten von allen. Darum wurde dieser Charakter als ein für diese Gattung zuverlässiger Diagnosenschlüssel angesehen. Man könnte ein genaueres Bild erhalten, wenn zukünftige technische Beschreibungen neben der Reichweite auch die Mittelwerte und die normale Abweichung enthalten würden. Diese Zahlen neigen dazu, die Wirkung der extremen Variationen auf eine Population zu reduzieren. Die Schwankung in der Länge war bis zu einem Viertel mehr oder weniger als der Mittelwert.

Nach dieser Angabe ist es offensichtlich, dass grosse Abweichungen vom Mittelwerte in jeder normalen Population zu erwarten sind. Man illustrierte eine Variation der Schwanzspitzenform weiblicher Exemplare von *P. zeae, P. penetrans, P. subpenetrans* und *P. hexincisus.* Die typische, kegelförmige Schwanz-

1) Translation for summary by Dr. Mark SCHWEIZER of the Department of Foreign Languages of the University of Maryland.

spitze von *P. zeae* kommt nur in etwa der Hälfte der untersuchten Exemplare vor, wogegen die anderen Exemplare in dieser Hinsicht eine geringe Variabilität aufweisen. Man fand in jeder Art solche, die gestreifte Schwanzspitzen hatten, ein charakteristisches Merkmal von *P. pratensis*.

In allen untersuchten Exemplaren von *P. zeae* fand man eine dorsale postrectale Verlängerung des Darmes, die früher im Genus *Pratylenchus* nicht nachgewiesen worden war. Das Vorhandensein dieser Erscheinung sollte in der technischen Beschreibung von *P. zeae* hinzugefügt werden, da auch Stücke aus der Nähe des typischen Fundortes die Darmmodifaktion zeigten.

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GENERALIA

I. ANDRÁSSY, Budapest VIII, Puskin-u. 3., Alltrendszertani Intézet, has lost a good deal of his nematological library. He would appreciate receiving recent reprints of papers on freeliving and plant parasitic nematodes.

P. A. A. LOOF and M. OOSTENBRINK. Plantenziektenkundige Dienst, Wageningen (The Netherlands) need the type species of: *Pratylenchus pratensis, Paratylenchus macrophallus* and *Macroposthonia annulata*. These types are lacking in DE MAN's collection and it is supposed that De Man had lent the slides to a colleague who did not return them. Would you kindly write to Mr. Loof, Wageningen if you know anything of the whereabouts of de Man's types or if you are able to procure co-types of the above mentioned species?

Change of address:

MEYL, A. H., now: Bad Godesberg (Bonn), Rüngsdorferstrasse 1a

A. H. MEYL

SOCIETY OF EUROPEAN NEMATOLOGISTS
FOURTH INTERNATIONAL NEMATOLOGY SYMPOSIUM

2. Information

Dear Colleague,

Below is more recent information about the Fourth International Nematology Symposium which takes place in Hamburg from the 5th to the 7th of September 1957.

The programme will be along the following lines:-

First day (5th September)

Morning: *Problems of Taxonomy*. Chairman Prof. Dr. B. G. Peters, London.

Afternoon: *Stem Eelworms*. Chairman Dr. P. Bovien, Lyngby.

Second day (6th September)

Morning: *Root Knot Nematodes*. Chairman Prof. Ing. J. van den Brande, Gent.

Afternoon: *Migratory Root Eelworms*. Chairman Dr. M. Oostenbrink, Wageningen.

Followed by the first full session of the Society of European Nematologists.

The meetings will take place in the Botanical Institute of Hamburg University, Jungiusstrasse 6-8, the Director, Prof. Dr. Mevius, having kindly placed the lecture room at our disposal.

On September 7th, an Excursion will take place in the largest area of Tree Nurseries in Europe which lies north of Hamburg. The cost per person (without meals) will be from 12 to 18 shillings.

Dr. H. GOFFART

Nematologica II (1957): 177-184

BEMERKUNGEN ZU EINIGEN ARTEN DER GATTUNG *MELOIDOGYNE* [1])

VON

H. GOFFART

Biologische Bundesanstalt, Institut für Hackfruchtbau, Münster/Westf.

Wurzelgallenälchen (*Meloidogyne* spp.) sind in Deutschland seit Jahrzehnten als Schädlinge vieler in Gewächshäusern kultivierter Pflanzen bekannt. Gelegentlich werden sie auch im Freiland, namentlich auf leichteren Böden, beobachtet, wo sie z.B. an Möhren, Sellerie und Schwarzwurzeln Wachstumsverzögerungen und Welkeerscheinungen hervorrufen. Ursprünglich wurden alle Wurzelgallenälchen zu einer Art unter dem Namen *Heterodera marioni* (Cornu, 1879) Goodey, 1932 zusammengefasst. CHITWOOD (1949) trennte sie dann auf Grund ihrer morphologischen und biologischen Eigenschaften von der Gattung *Heterodera* und fasste sie zur Gattung *Meloidogyne* zusammen. Weiterhin beobachtete er, dass sich die Wurzelgallenälchen morphologisch oftmals voneinander unterscheiden. CHITWOOD beschreibt 5 Arten und 1 Varietät und gibt den ersten Bestimmungsschlüssel. Inzwischen sind noch weitere Arten bezw. Varietäten beschrieben worden. Wir kennen heute folgende:

Meloidogyne exigua Goeldi, 1887
Meloidogyne javanica (Treub, 1885) Chitwood, 1949
Meloidogyne javanica var. *bauruensis* Lordello, 1956
Meloidogyne arenaria (Neal, 1889) Chitwood, 1949
Meloidogyne arenaria subsp. *thamesi* Chitwood, 1949
Meloidogyne incognita (Kofoid et White, 1919) Chitwood, 1949
Meloidogyne incognita var. *acrita* Chitwood, 1949
Meloidogyne hapla Chitwood, 1949
Meloidogyne brevicauda Loos, 1953
Meloidogyne inornata Lordello, 1956
Meloidogyne acronea Coetzee, 1956.

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1) Eingegangen: 17. Januar 1957.

Untersuchungen von Sasser (1954) zeigten, dass jede *Meloidogyne*-Art einen Wirtspflanzenkreis hat, der sich mit dem Wirtskreis anderer Arten zwar überschneidet, aber jede Art hat doch spezifische Wirte. Manche Kulturpflanzen, wie Kartoffel, Tomate und Zuckerrübe, werden von 5 Arten befallen. Da im Laufe der Jahre mehr als 2000 Kultur- und Wildpflanzen als Wirte von Wurzelgallenälchen bekanntgeworden sind, ist durch die neuen Erkenntnisse eine genaue Überprüfung des Wirtspflanzenkreises jeder Art notwendig geworden.

Aus Europa liegen bisher Untersuchungen aus Holland, Belgien und England vor. Oostenbrink (1955) fand — ausser einer noch unbekannten Art — *M. hapla, M. arenaria, M. javanica* und *M. incognita*. In Belgien treten *M. hapla* und *M. arenaria* vorwiegend in Blumen- und Gartenkulturen auf. Die Anwesenheit von *M. incognita* ist wahrscheinlich, aber noch nicht erwiesen (Gillard & van den Brande 1955). In England haben Brown (1955), Morgan (1956) und Stone (1956) *M. hapla* als Freilandschädling beobachtet. Offenbar kommen in Warmhäusern noch weitere Arten vor. So fand Southey (1957) kürzlich *M. arenaria, M. incognita* und *M. incognita acrita* an importierten Kakteen. Aus Deutschland liegen bisher ähnliche Untersuchungen nicht vor. Daher waren wir bestrebt, möglichst vielseitiges von Wurzelgallenälchen befallenes Material aus dem Freiland und aus Gewächshäusern zu erhalten, um zunächst einmal einen Überblick über die Verbreitung der Arten zu gewinnen und anschliessend Untersuchungen über den Wirtspflanzenkreis der Arten durchführen zu können.

Über die bisherigen Beobachtungen soll nachstehend berichtet werden. Ergänzend wurde auch Material aus dem Auslande herangezogen.

Technik

Für Untersuchungszwecke wurden die Weibchen aus dem befallenen Wurzelgewebe, das wenigstens 24 Stunden vorher in 5%igem Formalin gehärtet worden war, herauspräpariert. Bei genügend starker Infektion können auch befallene und fixierte Wurzelstücke klein geschnitten und mit etwa 80 bis 100 ccm Wasser in einen Mixer gebracht werden, der 10 Sekunden lang rotiert. Aus dem völlig zerkleinerten Material werden die herausgelösten Weibchen mit Pinsel oder Nadel entnommen oder in einem Sieb von 0,1 mm Maschenweite aufgefangen und einzeln mit einem winzigen Tropfen Glyzerin auf einen Objektträger gebracht. Der hintere Körperabschnitt wird dann vor-

sichtig unter dem Binokular mit einem sehr scharfen Skalpell vom übrigen Körper abgetrennt. Zweckmässigerweise entfernt man mit der Skalpellspitze auch den Körperinhalt des abgetrennten Hinterendes und dreht dieses so, dass es bei Betrachtung durch das Binokular in Aufsicht erscheint. Mehrere Hinterenden werden in dieser Weise auf einen Objektträger gelegt, mit einem Deckgläschen bedeckt und unter diesem vorsichtig ein Tropfen Laktophenol hinzugesetzt, der die Scheibchen umfliesst. So vorbereitet halten sich die Präparate für mehrere Monate in gutem Zustand. Dauerpräparate werden mit Glyceel umrandet.

Sämtliche Mikroaufnahmen wurden mit Ortholux Oc. 6 ×, Obj. 100 (Öl-Immersion) gemacht und sind nicht retuschiert.

Allgemeine Morphologie des Perineums und seiner Umgebung

Das Hinterende der Weibchen wird von Anus und Vulva bestimmt, die von einer teils niedrigen, teils hohen aus den Kutikularstreifen gebildeten Wölbung eingeschlossen werden. Die Begriffe „hoch" und „niedrig" machen gerade im Anfang bei einer Determination oft Schwierigkeiten. Wir versuchten daher, diese Begriffe zu präzisieren. Nach unseren Messungen, die sich auf mehr als 100 Weibchen erstreckten, wird man eine Wölbung dann als niedrig ansprechen können, wenn der Abstand von der Vulva bis zu dem Punkt, der noch eine deutliche Kutikularstreifung erkennen lässt, etwa das 2,3 fache der Vulvalänge beträgt (vgl. *M. hapla* und *M. arenaria*). Eine Wölbung gilt als hoch, wenn der vorgenannte Abstand etwa das 2,7 fache der Vulvalänge beträgt (vgl. *M. incognita*). Es dürfte zweckmässig sein, bei weiteren Untersuchungen darauf zu achten, ob diese Beziehungen konstant sind.

Die Kutikularstreifen gleichen in ihrer Linienführung dem Muster einer Fingerkuppe und variieren wie diese erheblich. Sie verlaufen in einem teils engeren, teils weiteren Abstand voneinander mehr oder weniger wellenförmig. Mitunter treten derartige Wellenlinien plötzlich an einer Stelle des Musters in gehäufter Form auf und werden dann wieder regelmässiger. Im Scheitelpunkt sind die Kutikularstreifen entweder abgerundet oder flach. Bei einer flachen Wölbung sind sie zuvor oft eingeschnürt, und der Scheitelpunkt kann sogar etwas eingesunken sein. Lateral sind sie unterbrochen oder ihr gradliniger Verlauf wird von kurzen Streifen abgelöst. Diese Unregelmässigkeit ist auf die Seitenlinie zurückzuführen, die hier die Kutikularstreifen

mehr oder weniger deutlich durchbricht. In der Nähe der oft unregelmässig angeordneten kurzen Streifen finden sich die Phasmiden, die als kleine glänzende Punkte seitlich oberhalb des Anus erkennbar sind. Kurze Kutikularstreifen treten auch an anderen Teilen des Perineums auf. So erstrecken sich manche im Verlauf der Seitenlinie bis in die Nähe der Schwanzspitze und hängen wie Fransen herunter. In der Umgebung der Schwanzspitze sind sie oft wirbelartig. Bei einer anderen Art tritt um die Schwanzspitze herum eine Punktierung auf, die sich sogar auf die lateralen und ventralen Teile des Perineums ausdehnen kann. Lateral sind die Kutikularstreifen in Höhe der Seitenlinien zuweilen schwingenartig entwickelt.

Die Vulva ist von endokutikularen, knotenähnlichen Gebilden (Bullae) umstellt, die vielfach durchscheinen, deren Funktion aber noch unbekannt ist. Linke und rechte Hälfte des Perineums sind einander nicht symmetrisch. Überhaupt gleichen sich in der Kutikularmusterung kaum zwei Weibchen, sodass man zur Identifizierung stets eine Reihe von Präparaten durchsehen muss. Gewisse Merkmale, die sich immer wiederholen, ermöglichen zwar eine Klassifizierung, daneben gibt es aber auch Muster, die z.Zt. noch mit keiner der bekannten Arten einwandfrei identifiziert werden können. Um Verwirrungen zu vermeiden, werden daher Muster, die mit den amerikanischen Typen in wesentlichen Punkten nicht übereinstimmen, derjenigen Gruppe, der sie am nächsten stehen, zugeordnet (z.B. *arenaria*-Gruppe). Dies scheint mir notwendig zu sein, da wir über die Variabilität der bisher bekannten Arten noch wenig unterrichtet sind.

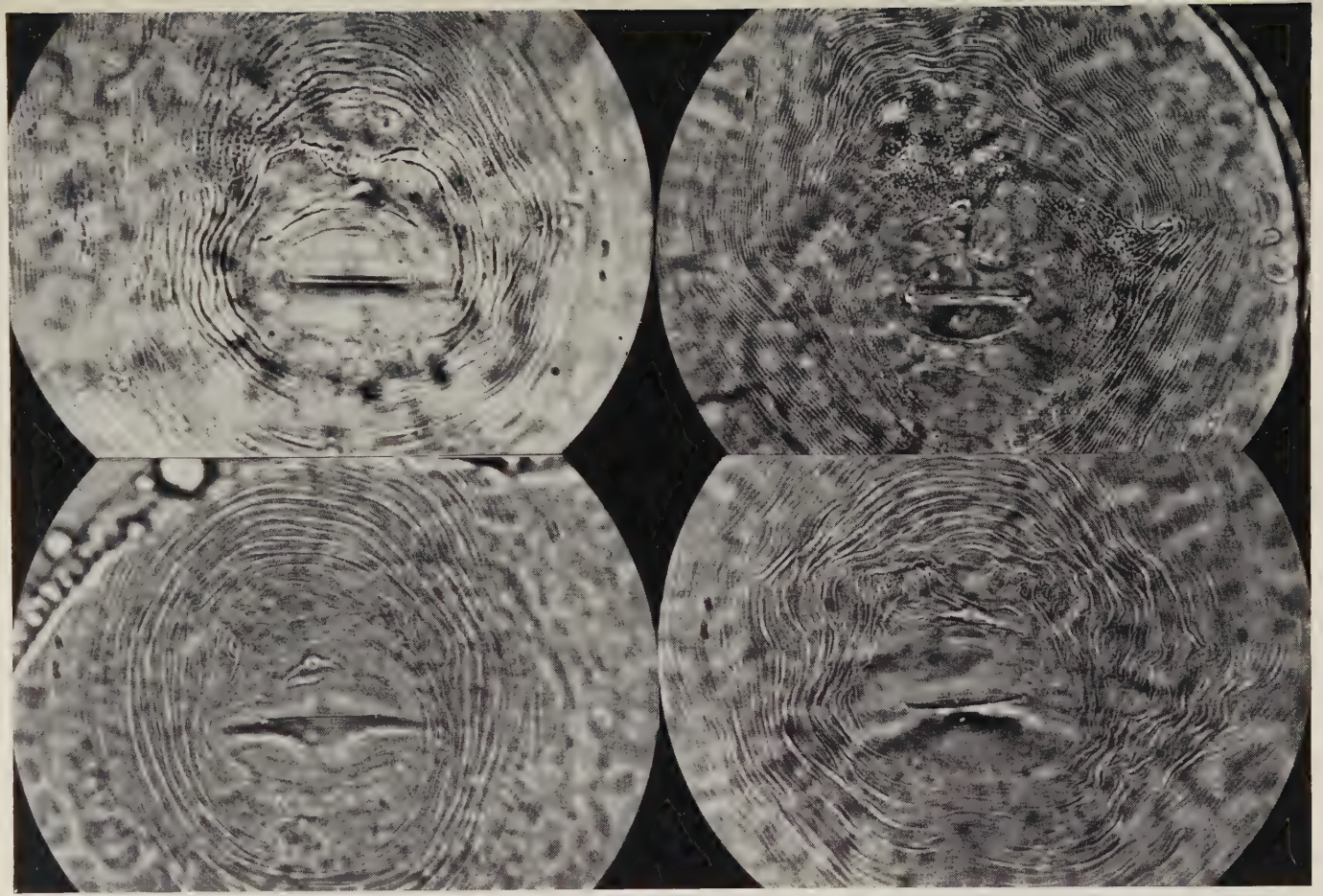
In vielen Fällen fanden sich an den untersuchten Pflanzen zwei und mehr Arten. Ob und inwieweit hierbei Bastardierungen vorkommen können, ist noch nicht untersucht worden.

Meloidogyne hapla Chitwood, 1949

Wölbung niedrig und rundlich (Abb. 1), gelegentlich auch dorsal abgeflacht und im Scheitelpunkt etwas eingesunken (Abb. 2). Seitenlinien sind nur durch Unregelmässigkeiten oder kurze Unterbrechungen der Kutikularstreifen angedeutet. Die Streifen selbst sind deutlich, jedoch durchweg nicht kräftig entwickelt, abgesehen vielleicht von den zentral verlaufenden. Alle Linien sind meist schwach wellenförmig ausgebildet. Die Streifen des dorsalen und ventralen Sektors können an der Seitenlinie einseitig oder auf beiden Seiten schwingenartig ausgebuchtet sein. Andeutungen einer solchen Entwicklung zeigt Abb. 2.

1

2

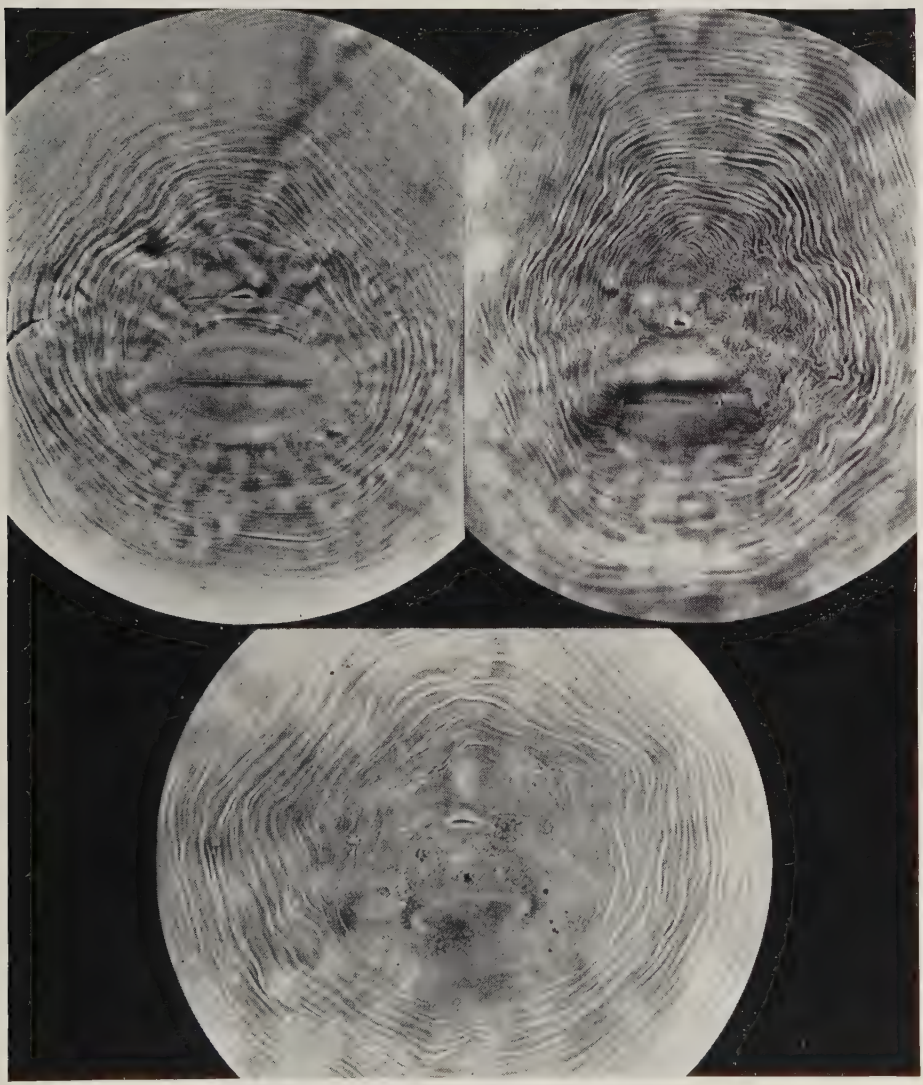


4

5

Abb. 1. *Meloidogyne hapla* von Rotklee (*Trifolium pratense*). — Abb. 2. *Meloidogyne hapla* von Tomate (*Lycopersicon esculentum*). — Abb. 4. *Meloidogyne arenaria* von Tomate (*Lycopersicon esculentum*). — Abb. 5. *Meloidogyne arenaria* von Tomate (*Lycopersicon esculentum*).

Abb. 6. *Meloidogyne arenaria* von Tomate (*Lycopersicon esculentum*) mit sehr unruhigem Muster. — Abb. 7. *Meloidogyne arenaria* subsp. *thamesi* von Gardenia (*Gardenia* sp.). — Abb. 8. *Meloidogyne arenaria* subsp. *thamesi* von Kaktus (*Cactus* sp.) mit sehr starker



11

10

Abb. 9. *Meloidogyne arenaria*-Gruppe von Zuckerrübe (*Beta vulgaris*). — Abb. 10. *Meloidogyne arenaria*-Gruppe von Zuckerrübe (*Beta vulgaris*) aus Infektionsversuch. — Abb. 11. *Meloidogyne incognita*-Gruppe von Tomate (*Lycopersicon esculentum*) mit starker seitlicher Kräuselung.

3

14

Abb. 3. Befall von *Meloidogyne hapla* an Möhre (*Daucus carota*). — Abb. 12. Befall von *Meloidogyne incognita*-Gruppe an Tomate (*Lycopersicon esculentum*). — Abb. 14. Befall von *Meloidogyne javanica* an Freesienknollen. Links ganze Knolle, rechts Schnitt durch eine Knolle, etwas vergrössert.

An der Vereinigungsstelle beider Seitenlinien nahe der Schwanzspitze erkennt man eine Stelle mit deutlicher Punktierung, die bei anderen Arten nicht beobachtet wurde und als charakteristisches Merkmal zu bewerten ist. Diese Punktierung dehnt sich mitunter auch auf die lateralen und ventralen Teile aus.

M. hapla tritt im Freiland allgemein auf und ist verhältnismässig leicht zu identifizieren. Sie bildet kleine Gallen, von denen Seitenwurzeln abgehen (Abb. 3). Folgende Pflanzen wurden angegriffen:

a) Kulturpflanzen: Möhre (*Daucus carota* L.), Salat (*Lactuca sativa* L.), Rotklee (*Trifolium pratense* L.), Phlox (*Phlox paniculata* L.), Tomate (*Lycopersicon esculentum* L.) und Rose (*Rosa* sp.).

b) Unkräuter: Kleiner Sauerampfer (*Rumex acetosella* L.), Windenknöterich (*Polygonum convolvulus* L.), Pfirsichblättriger Knöterich (*Polygonum persicaria* L.), Einjähriger Knäul (*Scleranthus annuus* L.), Schmalblättrige Wicke (*Vicia angustifolia* L.), Reiherschnabel (*Erodium cicutarium* d'Hér.), Kleinblütiges Vergissmeinnicht (*Myosotis arenaria* Schr.), Ackerminze (*Mentha arvensis* L.), Spitzwegerich (*Plantago lanceolata* L.), Kleinblütiges Franzosenkraut (*Galinsoga parviflora* Cav.) und Gemeine Schafgarbe (*Achillea millefolium* L.).

Meloidogyne arenaria (Neal, 1889) Chitwood, 1949

M. arenaria scheint eine wesentlich stärkere Variabilität als *M. hapla* zu besitzen. Bei *M. arenaria* typica ist die Wölbung niedrig und mehr oder weniger rund. Das Kutikularmuster ähnelt dem von *M. hapla,* es fehlt aber die Punktierung nahe der Schwanzspitze. Ausserdem verlaufen die Linien meist stärker wellenförmig. Eine Seitenlinie ist kaum festzustellen (Abb. 4). Gelegentlich wird sie aber durch Unregelmässigkeiten im Verlauf der Streifen angedeutet, die noch durch teilweise ungeordnete sehr kurze Streifen hervorgehoben werden können (Abb. 5). Durch den mitunter auf kurzer Strecke verstärkt auftretenden mit einem Kardiogramm vergleichbaren Linienverlauf, der übrigens auch bei anderen *Meloidogyne*-Arten beobachtet wurde, also keinesfalls für *M. arenaria* spezifisch ist, kann das Muster einen sehr unruhigen Eindruck machen. Dr. Franklin teilte mir mit, dass Phasmiden bei *M. arenaria* viel deutlicher als bei anderen *Meloidogyne*-Arten zu sehen sind. Sie liegen als glänzende Punkte unmittelbar

an den Seitenlinien wenig oberhalb des Afters, sind aber photographisch nicht recht zu fassen. Die Abb. 4, 5 und 6 stellen übrigens die an einer Tomatenpflanze beobachteten Muster dar. Sie zeigen deutlich, wie stark das Hinterende von *M. arenaria* bei derselben Wirtsptlanze variieren kann.

M. arenaria scheint vorwiegend ein Parasit in Gewächshäusern zu sein. Sie wurde an folgenden Pflanzen gefunden: Gummibaum (*Ficus elastica* Roxb.), Begonie (*Begonia* sp.), Zimmerlinde (*Sparmannia africana* L.), Gurke (*Cucumis sativus* L.) und Tomate (*Lycopersicon esculentum* L.).

Meloidogyne arenaria subsp. *thamesi* Chitwood, 1949

M. arenaria subsp. *thamesi* hat eine hohe, fast quadratisch aussehende flache Wölbung. Ein weiteres Hauptmerkmal sind die kurzen vertikalen Streifen, die wie Fransen herunterhängen (Abb. 7). Gelegentlich kommt auch hier eine ein- oder zweiseitig schwingenartig entwickelte Ausbuchtung des Kutikularmusters vor (Abb. 8).

M. arenaria subsp. *thamesi* konnte an folgenden Pflanzen nachgewiesen werden: Zuckerrübe (*Beta vulgaris* L.), Kaktus (*Cactus* sp.), Tabak (*Nicotiana tabacum* L.) und Gardenia (*Gardenia* sp.).

Neben diesen beiden Vertretern der *arenaria*-Gruppe gibt es aber auch Formen, die gewissermassen Kombinationen beider Typen vorstellen. So wurde in einem Falle eine sehr niedrige, dorsal jedoch deutlich abgeflachte Wölbung gefunden. Gut erkennbar waren auch die vertikalen Streifen (Abb. 9). In einem anderen Falle konnte eine niedrige fast rundliche Wölbung mit sehr deutlicher „Fransenbildung" beobachtet werden (Abb. 10). Beide Male scheint mir die Identifizierung mit *thamesi* etwas gewagt zu sein, da noch keine Übergangsformen beobachtet worden sind. Ausserdem stammen beide Aufnahmen von Wurzelgallenälchen an Zuckerrüben, und zwar Abb. 9 aus der Türkei, Abb. 10 von einem Freiland-Infektionsversuch mit Erde aus Deutschland. Um Verwirrungen zu vermeiden, werden sie daher zunächst in die *arenaria*-Gruppe eingeordnet.

Meloidogyne incognita (Kofoid et White 1919) Chitwood, 1949
Meloidogyne incognita var. *acrita* Chitwood, 1949

Ein in allen Teilen mit dem jeweiligen Typ übereinstimmendes Kutikularmuster konnte noch nicht aufgefunden werden. So weist Abb. 11 zwar eine hohe, aber auch deutlich abgeflachte Wölbung auf.

Lateral ist namentlich auf der rechten Seite eine beiderseits über die Seitenlinie erheblich hinausgehende Kräuselung zu erkennen, die anscheinend in dieser Form noch nicht beobachtet wurde. Erkennbar ist jedoch die für beide Typen charakteristische Gabelung der Streifen längs der Seitenlinie. Da genügend Vergleichsmaterial noch nicht zur Verfügung steht, scheint es zweckmässig zu sein, das Muster zunächst nur als zur *incognita*-Gruppe gehörig zu bezeichnen. Wurzelgallenälchen dieses Typs wurden im Gewächshaus an einer Tomatenpflanze gefunden. Sie bildeten hier grosse oft etwas längliche knollige Gallen (Abb. 12).

Meloidogyne javanica (Treub, 1885) Chitwood, 1949

Die Wölbung ist im allgemeinen niedrig und rund oder auch etwas abgeflacht; nach Taylor, Dropkin und Martin (1955) kann sie bei Varianten auch hoch sein. Charakteristisch sind die breiten gut erkennbaren Seitenlinien, die über die Kutikularstreifen oft noch hinausgehen (Abb. 13). Dadurch wird eine scharfe Trennung des dorsalen und ventralen Sektors erzielt.

M. javanica wurde in Deutschland bisher noch nicht gefunden, wird aber von Holland gemeldet (Oostenbrink, 1955), wo sie an Gurken unter Glas beobachtet wurde. Sie konnte jedoch an Wurzelmaterial von Tomaten aus USA, von Kartoffeln und Zuckerrüben aus der Türkei und von *Salix* aus Italien festgestellt werden. Bemerkenswert ist auch ein Auftreten in Freesienknollen, die aus Italien stammten. An der Aussenseite der Knollen zeigten sich nekrotische Stellen, die bis ins Innere zu verfolgen waren. Bei der Präparation stellte sich dann ein Befall durch *M. javanica* heraus. Abb. 14 zeigt die Symptome, die in dieser Form wohl noch nicht beobachtet wurden.

Herrn Dr. V. H. Dropkin, Nematode Research Laboratory, Seaford, N.Y. (USA) und Fräulein Dr. M. T. Franklin, Rothamsted Experimental Station, Harpenden (England) danke ich für einige Hinweise und Nachuntersuchungen.

SUMMARY

In the examination of root-knot nematodes occurring in Germany, the following species were found: — *Meloidogyne hapla, M. arenaria, M. arenaria* subsp. *thamesi. M. javanica* was also found in roots sent for examination. Some perineal patterns are described which previously had not been correctly identified. They belong to the *arenaria* and *incognita* groups. *M. hapla* occurs especially in fields and attacks many weeds. The species found in Germany are briefly described.

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RESISTANCE-BREAKING BIOTYPES OF THE POTATO ROOT EELWORM (*HETERODERA ROSTOCHIENSIS* WOLL.) [1]

BY

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The occurrence of biotypes of the potato root eelworm (*Heterodera rostochiensis* Woll.) capable of producing numerous cysts upon resistant clones of *Solanum tuberosum* subsp. *andigena* Juz. & Buk. [2]) and upon resistant seedlings bred from crosses of these clones with varieties of *S. tuberosum* subsp. *tuberosum* L. has been reported by VAN DER LAAN & HUYSMAN (1957), and DUNNETT (1957) and Anon (1956). In the first report, the eelworm population was obtained from Peru and in the second from a garden at Duddingston in Scotland. QUEVEDO, SIMON and TOXOPEUS (1956) have also shown that a Peruvian population of potato root eelworm was capable of breaking the resistance of 82 Dutch tuber-lines bred from C.P.C. 1673. Because of the importance of these findings, the writer undertook certain confirmatory tests at the request of the Agricultural Research Council.

Materials and Methods

Samples of infested soil were obtained from twenty localities and the cysts extracted from them. The Duddingston soil differed from the others in that it contained cysts that had been through one generation on a resistant plant. Where possible, the cysts were screened and only the larger sizes used. The egg contents of the cysts were estimated and, in a few cases, the numbers of hatchable larvae were also obtained. Small pots of 3½ in. diameter holding approximately 380 g. of soil were filled from a bulk of sterilized 4: 1 loam/sand mixture and sufficient cysts were introduced into each pot to give a level of infestation of 20 eggs/g. soil. The pots were sunk into gravel plunges, planted with tubers and watered liberally throughout the growing period.

1) Received for publication: February 16, 1957.

2) For shortness' sake called later-on in this paper: *Solanum andigenum.*

Periodically, the pots were turned out for visual assessment of the number of cysts on the external root mat. At the end of the experiment, the soil from each was floated and the number of cysts counted. On an average only 75 % of the old cysts originally added to the pots were recovered.

Results

Tables 1 and 2 give the results of an experiment in which cysts from five localities were tested against resistant plants derived from three

TABLE I

Tests of various resistant *S. andigenum* — *S. tuberosum* crosses against five local races of potato root eelworm. Cumulative results of three visual assessments of cysts on the peripheral root system

Source of Cysts		Duddingston, Midlothian	"B" Midlothian	Feltwell, Norfolk	Frampton, Lincs.	Rothamsted, Herts.
Resistant plants						
CPC. 1673 *line*						
2201(174)	*S. andigenum*	++++	—	+	+	—
2296.2(64)	F1 cross	++++	—	—*	—*	—*
Y2/26	F1 cross	++++	—	—	++	++
2371b(1)	B1 cross	++++	—	+	++	++
CPC. 1685 *line*						
NY27/3	F1 cross	++++	+	—	+++	+
Z3/7	F1 cross	+++	—	+	++	++
CPC. 1690 *line*						
H2/2	*S. andigenum*	+++	—	—	—	+
Y15/4	B1 cross	++++	—	—	++++	+
Control plants						
Potato (Arran Banner)		+++	++++	++++	++++	++++
Tomato (Klondyke Red)		+	+	+	+	+

Key — No cysts visible on external mat of roots.
\+ Up to 5 cysts visible.
++ 6-15 cysts visible.
+++ 16-50 cysts visible.
++++ Over 50 cysts visible.
* Grown from cuttings, development retarded compared with other plants.
F1 = *andigenum* × *tuberosum*
B1 = (F1 *andigenum* × *tuberosum*) × *tuberosum*.

TABLE II

Tests of various resistant *S. andigenum* — *S. tuberosum* crosses against five local races of potato root eelworm

Source of Cysts		Duddingston, Midlothian	"B" Midlothian	Feltwell, Norfolk	Frampton, Lincs.	Rothamsted, Herts.	
No. of cysts added per pot		52	42	50	56	54	
Estimated egg content		7,540	7,602	7,672	7,452	7,500	
Hatchable larvae in 21 days at 25°C		4,750	3,150	5,500	3,900	4,450	
		* *New cysts per pot at the end of the test*					
Resistant plants							
CPC. 1673 *line*							
2201	*S. andigenum*	1,047	5	0	79	0	
2296.2(64)	F1 cross	2,089	0	0	57	12	
Y2/26	F1 cross	677	5	3	117	1	
2371 b(1)	B1 cross	1,453	12	8	480	17	
CPC. 1685 *line*							
NY27/3	F1 cross	2,253	70	52	293	150	
Z3/7	F1 cross	1,673	19	23	318	117	
CPC. 1690 *line*							
H2/2	*S. andigenum*	** 355	19	14	44	18	
Y15/4	B1 cross	1,466	40	0	1,081	7	
Means for resisters		**1,377**	**21**	**13**	**309**	**40**	
% resistance breaking biotypes		*** 75	1	<1	17	2	
Control plants							Means
Potato (Arran Banner)		**** 310	1,615	2,272	593	2,790	**1516**
Tomato (Klondyke Red)		48	220	146	83	152	**130**

* New cysts = total cysts less 75% of the cysts added initially.
** H2/2 sprouted much later than the rest.
*** See table IV and page 190.
**** Tubers sprouted irregularly.

F1 = F1 *andigenum* × *tuberosum*
B1 = (F1 *andigenum* × *tuberosum*) × *tuberosum*

lines of *S. andigenum*. The plants were either *S. andigenum* selfed, first crosses or first back crosses with *S. tuberosum*. They were grown from tubers except for 2296.2.(64) which was grown from tubers and cuttings. Cultivated potato and tomato were included as controls. Visual assessments and cyst counts agree well except that the latter

TABLE III

Tests of the resistant *S. andigenum* — *S. tuberosum* cross, Z3/7, against various local races of potato root eelworm: cumulative results from three visual assessments of cysts on the peripheral root system

Source of Cysts		Tomato (Klondyke Red)	Potato (Arran Banner)	Z3/7 1	Z3/7 2	Z3/7 3
1st Batch						
Duddingston,	Midlothian	+	++++	+++	+++	+++
"B",	Midlothian	+	++++	+	+	—
Feltwell,	Norfolk	+	++++	—	—	—
Frampton,	Lincs.	+	++++	++	++	++
Rothamsted (1),	Herts.	+	++++	—	+	+
Shelford,	Cambs.	+	++++	++	+	+
2nd Batch						
Oxlode,	Isle of Ely	++	++	—	—	—
Cadishead,	Lancs.	+	+	+++	++	++
Albrighton,	Salop	+	++++	—	+	+
Colyton,	Devon	++	+++	+++	+++	+++
St. Leonards,	Sussex	+	+++	+++	+++	+++
Thornbury,	Gloucs.	+	+	—	—	—
Newcastle,	Northumb.	+	++++	+	—	—
Jersey,	Channel Isles	.	++++	+++	++	++
3rd Batch						
Ramsey,	Hunts.	—	++++	—	—	—
Rothamsted (2),	Herts.	+	++++	—	—	—
Fosdyke,	Lincs.	++	++	+	—	+
Deeping,	Lincs.	+	++	—	—	—
Scilly,	Scilly Isles	+	+++	—	—	+
Gulval,	Cornwall	—	++	++	—	—
Controls (1		—	—	—	—	—
(2		—	—	—	—	—
(3		—	—	—	—	—

Key: See foot of Table 1.

give more precise information. All resisters broke down in the Duddingston population and gave a large number of cysts, there was little break-down in population "B" or in the Feltwell populations, while break-down was intermediate in Frampton and Rothamsted populations. Tomato grown outdoors in small pots was far less efficient

TABLE IV

Tests of the resistant *S. andigenum* — *S. tuberosum* cross, Z3/7, against various local races of potato root eelworm

* New cysts per pot at the end of the test

Source of cysts		Tomato (Klondyke Red)	Potato (Arran Banner)	Z3/7 1	Z3/7 2	Z3/7 3	Means	% mean for potato
Batch I								
Duddingston,	Midlothian	30	1,412	1,570	1,035	687	**1,097**	75
"B"	Midlothian	70	1,636	0	12	0	**4**	<1
Feltwell,	Norfolk	222	1,341	6	0	0	**2**	<1
Frampton,	Lincs.	85	1,246	292	122	334	**249**	17
Rothamsted (1),	Herts.	169	1,727	11	122	21	**51**	4
Shelford,	Cambs.	223	1,418	72	37	75	**61**	4
Means		**133**	**1,463**					
Batch II								
Oxlode,	Isle of Ely	199	930	0	0	0	**0**	0
Cadishead,	Cambs.	120	935	573	805	483	**620**	73
Albrighton,	Salop	253	1,466	10	0	0	**3**	<1
Colyton,	Devon	146	824	364	742	476	**527**	62
St. Leonards,	Sussex	234	700	340	440	489	**423**	50
Thornbury,	Gloucs.	141	299	76	—	0	**38**	5
Newcastle,	Northumb.	157	777	29	28	5	**20**	2
Means		**179**	**847**					
Jersey,	Channel Isles	—	723	235	127	34	**132**	18
Batch III								
Ramsey,	Hunts.	52	716	0	6	0	**2**	<1
Rothamsted (2),	Herts.	36	373	0	0	10	**3**	<1
Fosdyke,	Lincs.	45	190	38	22	4	**21**	6
Deeping,	Lincs.	145	533	12	5	2	**6**	2
Scilly,	Scilly Isles	73	269	84	93	69	**82**	22
Gulval,	Cornwall	12	207	120	67	76	**88**	23
Means		**60**	**381**					
Controls (		1	1	0	1	0		
(Total (		3	0	0	2	0		
Cysts) (		1	0	3	1	2		

* New cysts = total cysts less 75% of the cysts added initially.

as a host than potato. The figures in Table 2 suggest that line 1673 confers slightly greater resistance than lines 1685 and 1690 and, more important, they also suggest that against Frampton cysts there is a greater degree of breakdown in the back-crosses than in the parental material and first crosses. Thus, towards these cysts, 2371b(1)

appears partially susceptible and Y15/4 completely susceptible with cyst counts of 480 and 1,081/pot respectively.

Tables 3 and 4 give the results of tests in which a first cross resister, Z3/7, was tested in threefold replication against twenty cyst populations. Not all the cysts were available at once and the experiment was set up in three separate batches. For the Jersey cysts the soil population was so low that insufficient cysts would have been provided had they been extracted. The tests in this case were done in the Jersey soil at an infestation level of 4 eggs/g. and set up at the same time as Batch II.

Again visual assessment agrees well with cyst counts and tomato is a less efficient host than potato under the conditions of the experiment. For each batch, the mean cyst population of the potato pots may be taken as the best measure of the number of potential females in the population, since the number of eggs introduced into the pots is approximately the same for all cyst populations. Similarly the mean cyst populations produced on the resister Z3/7 are a measure of the number of females of the biotype or biotypes capable of breaking resistance. A crude estimate of the percentage of these in each population is given in the final column in Table 4. The proportion ranges widely from less than 1 % to 75 %.

In order to check more widely the behaviour of the various populations of potato root eelworm, tests of the same type were done with the following Solanaceous plants except that in this experiment the plants were kept indoors from July to November: —

Browallia grandiflora R. Grah.
Datura tatula L.
Lycopersicon peruvianum Mill.
Nicandra violacea André
Nicotiana affinis Moore
Nicotiana glutinosa L.
Nicotiana rustica L.
Nicotiana tabacum L.
Physalis angulata L.
Saracha umbellata G. Don.
Schizanthus pinnatus Ruiz & Pad.
Scopolia lurida Dun.
Solanum capsicastrum Link.
Solanum nigrum L.
Solanum nodiflorum Tag.
Solanum sisymbrifolium Lam.

Visual examination of roots and counts of cysts did not provide evidence that any population was capable of multiplying freely on these plants. This does not rule out the possible formation of a few cysts on some of the plants which might be overlooked by both methods of examination. Under the same conditions, cysts formed on the root

systems of tomato and *Solanum demissum* Lindl. although the latter grew very poorly.

Discussion

Resistance to potato root eelworm in *S. andigenum* was first demonstrated by Ellenby (1952). Later work in Holland (Toxopeus and Huysman 1952, 1953; Huysman 1955, 1956), Scotland (Anon 1956), England (Jones 1954, Howard 1955, 1958) and Northern Ireland (Howard *in litt.*) demonstrated that resistance was due to a dominant gene designated H. The limited survey described above indicates a fairly wide occurrence of resistance-breaking biotypes in the United Kingdom and suggests that, by chance, all the above work was done with potato eelworm populations containing few eelworms of this type. Thus the work at Cambridge was done with soil from Feltwell Fen, Norfolk and the Scottish work with soil from site "B".

Huysman (1955, 1956) has discussed the significance of the few cysts appearing on the roots of resistant plants. His test population produced rather more cysts on line 1685 than on line 1673. The limited data in Table II support the view that resistance in line 1673 is stronger. Huysman also found no evidence of diminution of resistance in backcrosses. Table II, however, suggests that in some populations of eelworm a partial or complete breakdown may occur although the evidence based solely on two backcrosses in a single test is rather slender.

The author's tests confirm in detail the findings of Dunnett (1957). Further work is now necessary on backcrosses and on the mode of inheritance of resistance-breaking in potato root eelworm itself. The latter has a bearing on the rate at which the proportion of the biotypes in natural populations will increase when 'resistant' potatoes are grown (i.e. potatoes on which few or no cysts are produced when they are grown in soils with eelworm populations similar to those found, for example, at Feltwell and site "B"). If resistance-breaking behaves as a simple dominant, increase would probably be rapid; if recessive very slow at first. Whatever the mode of inheritance, the rate of increase of the biotypes is likely to be slower than with new strains of potato blight (*Phytophthora infestans* de Bary) because of the lack of mobility of eelworms compared with air-borne fungal spores and because of the relatively slower rate of reproduction.

I wish to thank Dr. W. Black, Mr. J. M. Dunnett and Dr. H. W.

Howard for supplying resistant *S. andigenum* hybrids and for many helpful suggestions. Thanks are also due to Dr. W. Black, Mr. J. M. Dunnett and members of the N.A.A.S. for sending soil samples and to Miss Joan Evetts and Miss J. M. W. Hurrell for technical assistance.

ZUSAMMENFASSUNG

Zwanzig Populationen von Kartoffelnematoden (*Heterodera rostochiensis*) wurden geprüft, ob unter ihnen Biotypen auftreten, die imstande sind, die Resistenz von *Solanum tuberosum* subsp. *andigena* und von Kreuzungen dieser Unterart mit *Solanum tuberosum* zum Zusammenbruch zu bringen. Derartige Biotypen sind im Vereinigten Königreich ziemlich weit verbreitet. Die Linie 1673 hatte gegenüber 5 Lokalrassen des Kartoffelnematoden eine grössere Resistenz aufzuweisen als die Linien 1685 und 1690. Bei den geprüften Populationen lag der Prozentsatz der agressiven Weibchen zwischen < 1 und 75 %. Eine Lokalrasse des Kartoffelnematoden zeigte eine stärkere Agressivität bei Rückkreuzungen als bei den Eltern oder der ersten Kreuzung.

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PREDATION OF NATURAL POPULATIONS OF FREE-LIVING NEMATODES BY FUNGI [1])

BY

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There is no direct evidence that fungi predacious on nematodes are active in forest litter, but there is ample evidence that many species of predacious fungi occur in this type of habitat. Observations on the occurrence and behaviour of predacious fungi of free-living nematodes in soil habitats have been confined mainly to nutrient agar cultures (Drechsler 1941; Dollfus 1946). Duddington (1955) pointed out that it is dangerous to draw conclusions from agar cultures about the behaviour of fungi under natural conditions. Four observations of nematode predation by fungi under natural conditions have been reported. Zopf (1888) found that eelworms isolated from dung were frequently infected with the hyphae of *Arthrobotrys oligospora* Fres. Rahm (1922) observed that free-living nematodes of moss cushions are captured by *A. oligospora* and *Harposporium anguillulae* Lohde. *A. oligospora* has also been observed to capture the parasitic nematode *Anguillulina dipsaci* (Kühn, 1857) [3]) amongst the tissues of diseased leaves of *Saxifraga cotyledon* L. and *Calceolaria integrifolia* L., in a manner which was the same as that observed in the laboratory (Goodey 1938). Linford and Yap (1939) in their pot experiments upon the biological control of the root knot nematode of pineapple found the trapping mechanisms and mycelia of several species of predacious fungi on *Heterodera marioni* (Cornu, 1879) Goodey, 1932, and other nematodes washed from soil samples. We have found that a small proportion of nematodes isolated directly from forest litter has been preyed upon by fungi. The various forms of fungal predation which we have encountered so far are described in this account.

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1) Received for publication: February 26, 1957.

2) Now at The United Steel Companies Ltd., Sheffield, England.

3) Now named: *Ditylenchus dipsaci* (Kühn) Filipjev.

Isolation procedure and technics

Nematodes were isolated by two alternative techniques from samples of litter collected at random below a stand of high oak wood in Roudsea Wood Nature Reserve, Lancashire (Capstick unpublished). The particular litter horizon investigated is the F layer, which is composed of partly decomposed plant remains that have not completely lost their original form.

Isolation techniques and the examination of nematodes

(a) Bulked samples of litter were mixed with water, the larger fragments of litter removed with a 30 mesh/inch sieve (British Standard 410/1943), and the nematodes then retained on another sieve of 300 mesh/inch (B.S. 410) and recovered by washing the sieve with water.

(b) Nematodes were extracted in water from separate aliquots of litter shaken intermittently for periods of 24 or 48 hours (Minderman 1956). The nematodes were recovered on Whatman No. 50 filter paper in a Hartley funnel.

The nematodes recovered from the sieved samples were heat relaxed at 50° C for 5 minutes and were then fixed with formol — acetic fixative (Goodey 1951). Specimens extracted in the shaker were stained while still on the filter paper. All specimens were stained with a hot solution (60° C) of cotton blue in lactophenol for 3-5 minutes. Specimens which showed normal morphology and no signs of decomposition were mounted in lactophenol (Capstick 1956). They were identified (C.K.C. & D.C.T.) and later examined (J.S.W.) for the presence of fungi. The nomenclature of the nematodes isolated is according to Goodey (1951a).

Observations of fungal predation

Hyphae were seen penetrating the integument of a variety of species of eelworm. The most frequent type of infection consisted of apparently aseptate hyphae with a diameter of less than 1 μ. Details of the species of nematode infected are shown in Table I.

Hyphal snares

Five nematodes, a Tylenchid, two specimens of *Teratocephalus terrestris* (Bütschli, 1873) de Man, 1876, and single specimens of *Alaimus primitivus* de Man, 1880, and of *Wilsonema otophorum* (De Man, 1880) Cobb. 1913 (Fig. 1) were each ensnared by a

TABLE I

Hyphal infections of nematodes

Type of hyphae	*Nematode species (and no. observed)*	
External hyphae less than 1μ in diameter, aseptate.	*Bunonema reticulatum* Richters, 1905	1
	B. richtersi Jägerskjöld, 1905	4
	Teratocephalus crassidens de Man, 1880	1
	Triplonchium cylindricum Cobb, 1920	3
	Wilsonema otophorum (de Man, 1880) Cobb, 1913	1
	Tylenchid	1
	Unknown species	2
External hyphae, greater than 1μ in diameter, aseptate.	*Bunonema richtersi*	3
	Plectus sp.	1
External hyphae, greater than 1μ in diameter, septate.	*Mononchus* sp.	1
	Plectus sp.	1

constricting ring consisting of three swollen cells. The specimen of *W. otophorum* (285 μ long) had been trapped by one of four rings borne on a solitary hypha. Of the three empty rings two had been sprung without capturing prey. The other four specimens were each more than 350 μ long and they had managed to wrench their snares away from their anchoring hyphae. It is likely that they would have been killed before the invasion of their bodies by trophic hyphae from the encircling ring (DUDDINGTON, *in litt.*). The mean diameter of the closed rings was 20.6 μ (range 14-27 μ) and the mean diameter of their swollen hyphal cells was 7.8 μ (range 5-10 μ). The stalk cells were 2.0-2.5 μ in diameter and the stalk of the open ring was 11.5 μ long. The latter had a total diameter of 23 μ.

Spore forming fungi

I) *Harposporium oxycoracum* DRECHSLER (1941a).

Five specimens of *Bunonema reticulatum,* four specimens of *B. richtersi* and an unidentified *Aphelenchoides* were infected by a fungus forming globular cells borne on conidiophores developed from the carcasses of the infected nematodes. The morphological features (see Fig. II, 1, 2, and 3) agree well with those described for the imperfect fungus *Harposporium oxycoracum* Drechs., (Table II) with the exception of the measurements of spore length which are less than those given by DRECHSLER. This discrepancy is almost certainly due to the fact that we could only measure the length of spores that had not

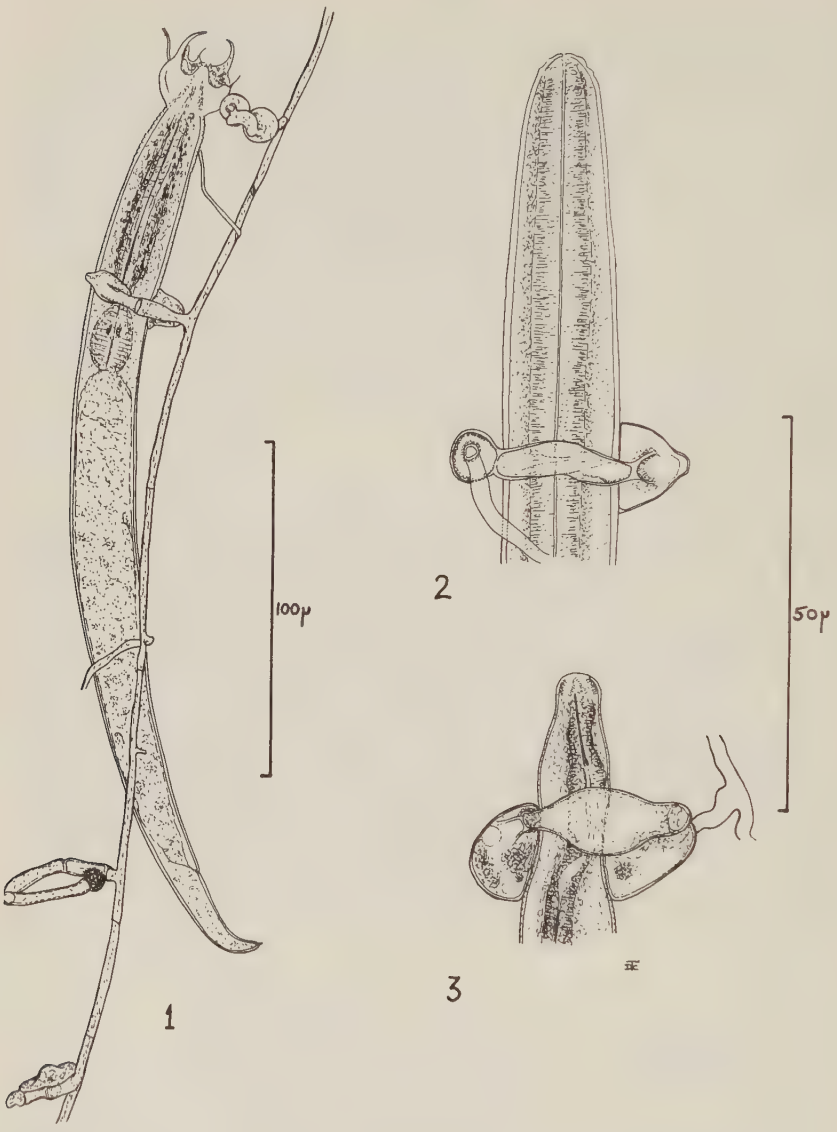

Fig. 1. Nematodes trapped by constricting rings. (1) *Wilsonema otophorum*; note sprung ring near anterior end of the eelworm. (2) *Alaimus primitivus*. (3) Tylenchid.

been shed from the globular cells and which were in consequence immature (Table II).

II) An infection seen on an unidentified worm had morphological

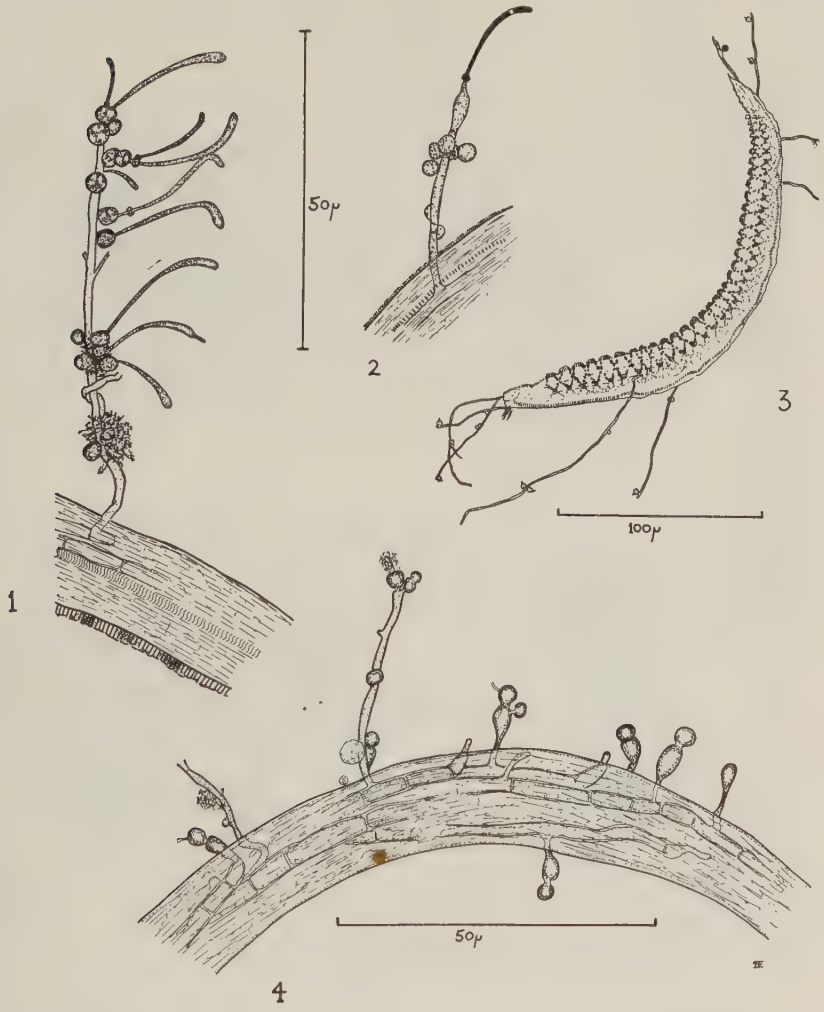

Fig. 2. Nematodes infected by sporing fungi. (1) *Bunonema reticulatum*; detail of conidiophore of *Harposporium oxycoracum* showing globular cells and conidia. (2) Ditto with single conidium. (3) *B. reticulatum* infected by *H. oxycoracum* showing at least seven conidiophores. (4) Unknown worm infected by a *Harposporium*(?) sp. Note globular cells with sterigmata and well defined mycelium in carcass.

features (Fig. II, 4) resembling the developmental stages of a *Harposporium* species reported to be *H. anguillulae* Lohde by KARLING (1938). The hyphae within the nematode were septate, 2.5 μ wide, in segments from 5 to 13 μ long. The conidiophores ranged in size

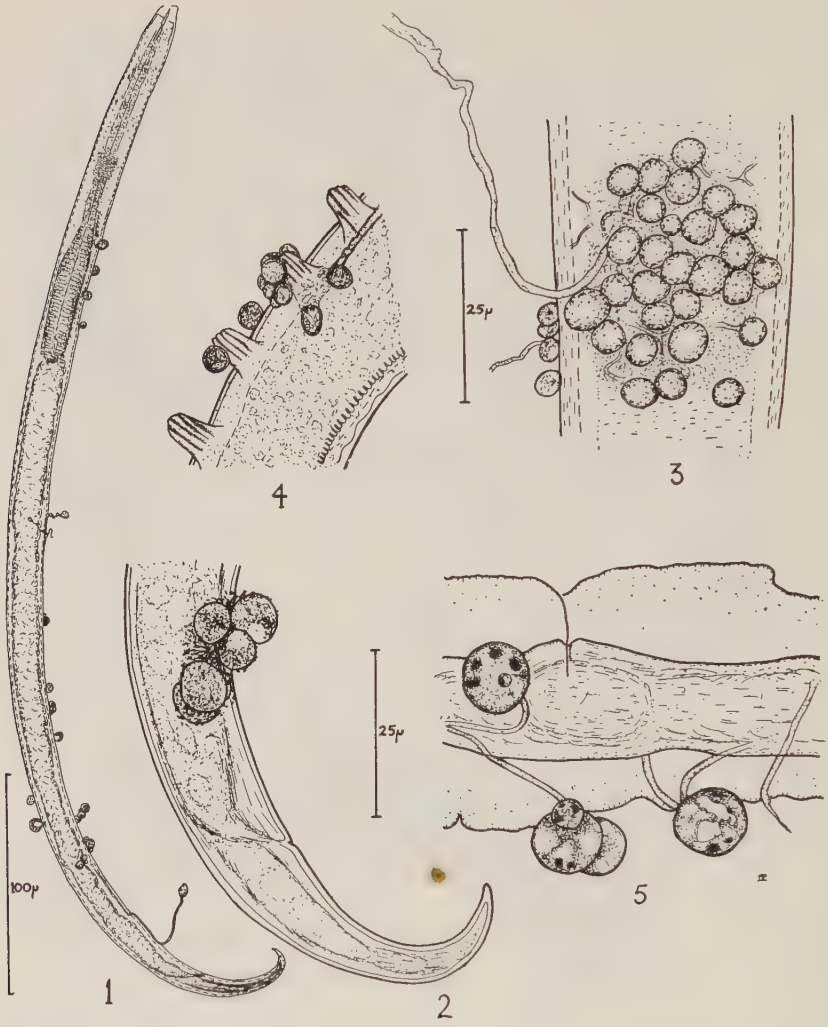

Fig. 3. Spherical bodies found on surfaces of nematodes. (1) *Dorylaimus parvus* bearing many spheres, some with hyphae. (2) Aggregation of spheres near tail of a *Dorylaimus parvus*. (4) *Bunonema richtersi*. (5) Decomposed *Plectus* sp. infected by aseptate hyphae bearing large spheres.

from 3 to 37 μ long and were from 2 to 3 μ wide, with a narrow basal region about 1 μ wide. The globular cells were 3.2 μ (2.0-5.0 μ) in diameter, but mature spores were not seen.

III) A fungus with fertile hyphae on a specimen of *Bunonema richtersi* had formed conidiophores as long as 185 μ which were 2-3 μ wide at the base, narrowing to 1.5 μ at their apices. The phialides

TABLE II

Comparison of *Harposporium oxycoracum* Drechsler with *Harposporium* sp. from Roudsea Wood.

	Harposporium oxycoracum	*Harposporium* sp.
	On maize-meal agar plates inoculated with leaf mould, Wisconsin, U.S.A. (DRECHSLER 1941a).	Infecting nematodes in leaf litter from Roudsea Wood, 1956.
Conidiophores	1-3μ × 25-1000μ	1.8 (1.5-2.5μ) × 15-117μ
Globular cells	3.5-4.5μ	3.6 (2-5μ)
Sterigmata	0.8μ × 1.2-2.0μ	0.5 × 1-3μ
Conidia	0.7 - 1.4μ × 15-25μ	1μ × 14.2μ (7-20μ)
Basal knob on conidium	0.5 - 1.0μ wide	± 1μ wide

were slender from 14 to 20 μ long, tapering to 1.5 μ at their apices, where solitary spores were being formed. The largest spore observed was 3.5 μ long and 2.0 μ wide. On these inadequate data it is not possible to assign this fungus to any genus of the imperfect fungi.

Spherical structures on the integuments of nematodes

The main morphological features of a variety of spherical bodies found on the surfaces of nematodes are shown in Table III. Most of these spheres were attached to the worms by hyphae; this, and the lack of internal pigments or structure apart from vacuoles, is a strong indication that these infections were fungal. We hope to identify the fungi concerned by isolating them in culture from infected worms. We have not found records of similar infections of free living nematodes in soil or litter.

SUMMARY AND CONCLUSIONS

A small proportion of samples of free living nematodes isolated directly from forest litter were found to be infected with various kinds of fungi. The present results confirm that predacious fungi are active in a natural population of nematodes from a soil habitat, and we hope to publish quantitative studies of this activity.

The more common infections were by hyphae or by small spherical bodies (from 2.5 to 13.0 μ in diameter), often associated with hyphae.

TABLE III

Spherical bodies found on surfaces of nematodes

Nematode species (and no. examined)	*Mean dimensions μ (and size range of spherical structures)*	*Notes*
Dorylaimus parvus de Man, 1880. (4)	5.4 (3-8)	Some attached to worm by hyphae from 1 to 1.5μ wide. Hyphae are aseptate cytoplasmic avacuolate. Some of the spheres contain vacuoles. ± Aggregation of spheres. (Fig. III, 1 & 3).
Plectus sp. (3)	5.8 (3-8.5)	Ditto.
Bunonema richtersi (1)	3.6 (2.5-4)	Vacuole in cytoplasm, no hyphae. (Fig. III,4)
Teratocephalus crassidens (1)	7.0 (6-8)	Hyphae present.
Alaimus primitivus (1)	8.3 (8-8.5)	Hyphae present.
Prismatolaimus sp. (1)	4.8 (4-7)	Hyphae present.
Unknown species (2)	6.3 (2-9)	Aggregated in one specimen, no hyphae.
Plectus sp. (1)	10.2 (6-12)	On aseptate hyphae 2μ diam. which penetrate the carcass in a group (Fig. III,5)
Dorylaimus parvus (1)	12.3 (7-13.5)	Aggregated (Fig. III, 2).

Some nematodes had been trapped by hyphal snares of the constricting ring type. A number were infected with spore-forming fungi, including *Harposporium oxycoracum*. Apart from this species, identification of the fungi has not so far been possible.

ACKNOWLEDGEMENTS

The authors are very grateful to Dr. V. M. Conway, Dr. C. L. Duddington and Dr. Owen Gilbert for reading and criticising the manuscript.

ZUSAMMENFASSUNG

Ein kleiner Teil aus Waldstreu isolierter freilebender Nematoden war von verschiedenen Pilzarten infiziert. Die Ergebnisse bestätigen, dass räuberisch lebende Pilze in einer natürlichen Nematodenpopulation sehr aktiv sind. In Kürze sollen quantitative Untersuchungen dieser Aktivität veröffentlicht werden. Die häufigste Infektion erfolgt durch Hyphen oder kleine, runde Körper (2,5 bis 13 μ im Durchmesser), die oft mit Pilzen in Gemeinschaft leben. Einige Nematoden fangen sich in Schlingen, andere werden von sporenbildenden Pilzen, einschliesslich *Harposporium oxycoracum,* infiziert. Abgesehen von dieser Art war eine Identifizierung der Pilze bisher nicht möglich.

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A CULTURE METHOD FOR SOIL MEIOFAUNA AND ITS APPLICATION TO THE STUDY OF NEMATODE PREDATORS [1])

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Laboratory culturing of the soil meiofauna has been a problem for many years, and the lack of suitable techniques, a serious hindrance to progress. Existing methods have been developed primarily for life-history and biological studies of the Acarina and Collembola, the commonest members of the soil meiofauna. Some have been used in investigations of anoplocephaline cestodes, parasites of sheep, of which oribatid mites are intermediate hosts, and recently modifications have been devised by those concerned with trombiculid mites, important because of their connection with the transmission of 'scrub-typhus'. Some of the culture methods described here offer a means for biological studies of certain nematodes, and the observations on insect predators of soil and plant-parasitic nematodes outlined, demonstrate the possibilities of these culture techniques.

The minimum requirements for culturing meiofauna are a suitable vessel and cover, and a substrate which will retain sufficient water to provide the humid environment normally required by these creatures. MICHAEL (1884), in his studies of the Oribatoidea, used a cell formed from a glass ring cemented to a microscope slide, with a cover glass as lid, and a circle of filter-paper on the floor of the cell to provide the necessary reservoir of water. This method has been widely used in studying the Acarina and other organisms. For culture of cheese mites (Acaridoidea), ROBERTSON (1945) used a plastic strip in which a hole with inclined sides was drilled. Black filter-paper was cemented to the lower surface, and the upper was sealed with a cover glass held in position by a paraffin-vaseline mixture. This pattern can be suspended in a vessel containing a suitable solution, and in this way the relative humidity of the cell atmosphere can be controlled precisely. CUNNINGTON & SOLOMON (*in litt.*) have modified the Robertson cell

1) Received for publication: March 4, 1957.

by replacing the filter-paper with 200-mesh bolting silk dyed black, and by securing the cover glass with ticket clips. JONES (1951) used a further modification with which he cultured the harvest mite (*Trombicula autumnalis* Shaw) from larva to adult for the first time. His pattern consisted of a perforated plastic block with filter-paper fastened to the lower surface. The chamber was formed from a strip of filter-paper resting in the cavity, its ends being held in position by a cover glass placed on the upper surface of the perspex. A cotton-wool plug was inserted between the two pieces of filter-paper, and the cell placed in a larger vessel, its lower surface in contact with moist cotton wool.

Plaster of Paris has also been used to provide suitable humidity conditions. For culturing Trombiculidae — difficult creatures to rear and a good test of technique — MICHENER (1946), JENKINS (1947), LIPOVSKY (1953) and others, used a jar with the bottom removed, and replaced by a plug formed from a mixture of plaster of Paris and activated charcoal (JENKINS 1947). To prevent the formation of water droplets, the walls were also coated with this material (MICHENER 1946). Recently EDWARDS (1955) has used a modification for culturing Symphyla, in which the cells are formed from glass cylinders embedded in plaster of Paris. ROHDE (1956) has devised a somewhat similar modification for phthiracarid mites. SCHALLER (1953) and his students, used a cell formed entirely of plaster of Paris with a glass cover seated in plasticine. This receptacle has the advantage that water can be added from outside without disturbing the cell contents. It has proved suitable for a wide range of animals including Diplopoda, Acarina and Collembola. SCHUSTER (1956), for biological studies of soil-inhabiting Oribatoidea, used a porous clay pipe-filter placed on a surface of moist sand.

None of these methods is entirely suitable for studies requiring close observation of the culture subject, and for this reason the first author has developed a new technique, which has proved very suitable for this purpose. The second author has also found it a useful container for electronic-flash photomicrography of living material (Figures 5 & 6) and for studying predators of some nematodes, chiefly the Heteroderidae. In addition to the basic requirements — high humidity, adequate oxygen supply, food material maintained in a moist condition, and absence of water droplets — such studies require a culture receptacle with a small surface area, and reduction to a minimum of wall surfaces and other awkward parts, which make observation dif-

ficult. For studies of food habits particularly, the presence of filter-paper, plaster of Paris or a loose substrate such as sand (Franz & Leitenberger 1948) or vermiculite (Farrell & Wharton 1949), is undesirable. A completely inert, solid medium has the further advantage that sterilization of the cell becomes a comparatively simple operation.

Method

The culture chamber consists of a sintered-glass micro-immersion filter [1]) (Fig. 1) with sunk disk; it has an area of 64 mm² (9 mm *d*), is 2 mm deep, and has a cover-glass lid. The glass syringe has a cotton-wound plunger. The filter is attached by rubber tubing

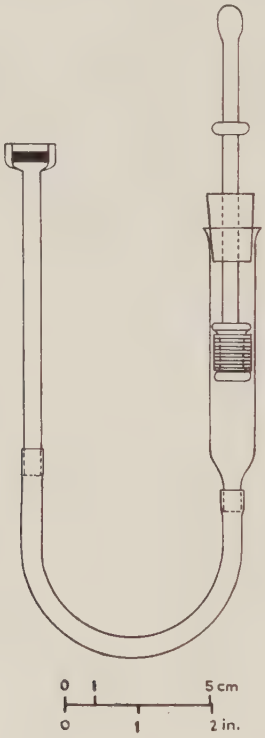

Fig. 1. Micro-immersion-filter culture cell with syringe.

1) The micro-immersion filters (K902H) were obtained from Messrs. Baird & Tatlock (London) Ltd., Freshwater Road, Chadwell Heath, Essex, England. They are available in two porosities: No. 1 (pore size: 90-150 μ) and No. 2 (40-90 μ). No. 2 is preferable as the surface of the former is rather coarse.

to the syringe, and is mounted in a suitable framework (Fig. 2). The system is filled with water, and when the plunger is depressed it is possible to irrigate the cell; on withdrawing the plunger, the water can be brought below the level of the sintered disk, and no water droplets should remain on the glass parts of the cell. The only troublesome feature is condensation on the cover. Avoidance of large temperature fluctuations and a fairly thick glass for the lid reduce condensation to a minimum. Demisting compounds also help.

There is frequently need for a larger culture vessel (e.g. for stock cultures) and for this purpose a Gooch-type filter crucible [2]) with sintered-glass plate (Fig. 3) is used. The crucible is inverted, inserted in a cork with a tube connected to a large syringe of the type already described. This arrangement provides a very shallow cell with a large surface area (27 mm $d \times 2$-3 mm deep; area = 573 mm^2). It is covered with a large cover glass, and the lower edge of the crucible is ground with carborundum powder if a tight seal is required. Alternatively, if there is need for a deep cell (4 cm) the crucible is turned right way up and inserted in a rubber cone adapter. This arrangement is particularly suitable for collecting live material from litter and soil samples when a funnel-type extractor is used.

It is important to ensure that the glass walls of the cell are thoroughly clean as a dirty surface traps water droplets during irrigation. For this reason cells should be cleaned in dichromate solution and then rinsed in distilled water.

Handling individuals of the soil meiofauna is a slow and tedious operation. Immersion in water for a short period does not harm some groups (e.g. the majority of the Oribatoidea) and this provides a convenient means for transferring live material. The animals are collected from a funnel extractor in a deep Gooch-crucible cell. The latter is flooded until the water reaches the upper rim of the cell, and the individuals, which usually float on the water surface, are removed with a fine brush, and placed in a miniature cell flooded in a similar manner. When the required number have been transferred, the water is withdrawn, thus completing the preparation of the culture.

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2) The Gooch filter crucibles (a-4840, 1 × porosity 1 = 100-120 μ) and matching rubber cone adapters were supplied by Messrs. A. Gallenkamp & Co. Ltd., 17-29 Sun Street, London, E.C. 2. The crucible pattern may vary slightly, especially the depth of lip which forms the cell wall. For this reason it is worth obtaining a sample before ordering in quantity.

Studies on animal predators of the Heteroderidae and other soil nematodes.

In July and August, 1956, while examining rape seedlings from a plot on which the cabbage-root nematode (*Heterodera cruciferae* Franklin) had been cultured for about seven years, it was found that whereas moderate infestations of larvae up to late third or early fourth stage occurred in the roots, the number reaching maturity appeared rather low. Moreover, of the few mature females found attached to the roots, sometimes up to about 30 per cent. had large irregular perforations through the cuticle and the body contents had usually disappeared (Fig. 4).

Although perforations of various forms have often been noticed in *Heterodera* cysts, so far as the writers are aware, no animal predators causing this type of damage have hitherto been identified. THORNE, however, reported *Mononchus papillatus* Cobb on two occasions attacking and drawing out the body contents of a young female *Heterodera schachtii* Schmidt, which was attached to a rootlet. He and other workers have also frequently observed *Mononchus* species feeding on mature males and larvae of *H. schachtii* and other soil nematodes and also attacking the eggs of *H. schachtii* in cysts, which had been opened, presumably by artificial means (THORNE 1927). THORNE (1928) also listed a number of species of *Acrobeles* and *Cephalobus*, *Dorylaimus obtusicaudatus* Bastian, *Tylenchus* sp. and *Plectus* sp. which he had found inside cysts of *H. schachtii*. Three *D. obtusicaudatus* were found with eggs of the sugar-beet nematode impaled on the mouth stylet, but as this nematode is not usually predacious, THORNE concluded that these occurrences were probably accidental. *Acrobeles* and *Cephalobus* are probably saprozoic and were not considered likely predators of eggs or larvae of *H. schachtii*.

Amongst others, J. J. HESLING and J. F. SOUTHEY (*in litt.*) have found species of *Dorylaimus* within *Heterodera* cysts, and these occurrences have sometimes coincided with the presence of a small circular perforation in the cyst wall. Although it seems probable, it has not been definitely established that these nematodes were preying on the living cyst contents.

Other soil-inhabiting organisms have been reported preying on heteroderas: SCHAERFFENBERG and TENDL (1950, 1951) concluded from experiments that young enchytraeids of the genera *Fridericia* and *Enchytraeus* fed on larvae of *H. schachtii* within the host plants, prob-

Fig. 2. A battery of micro-immersion-filter culture cells. A separate filter is shown in the forground.

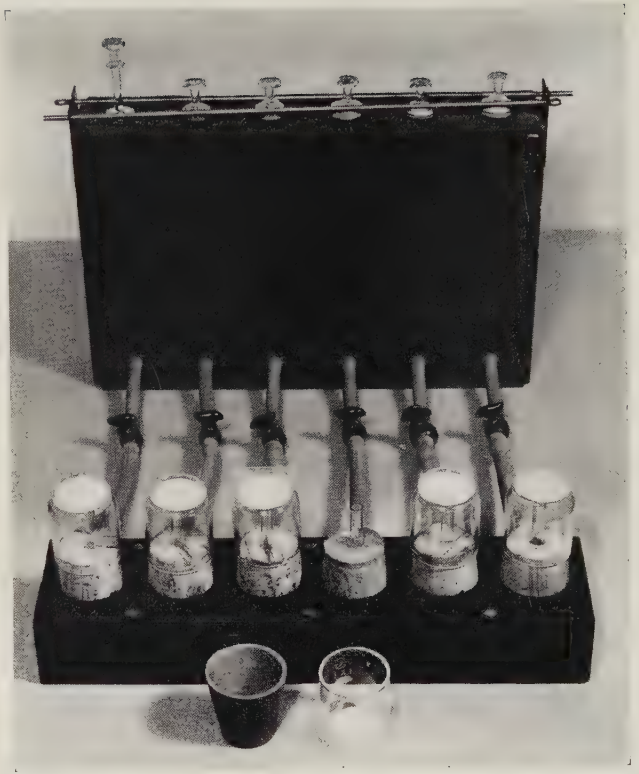

Fig. 3. Gooch filter-crucible cells with rubber cone adapter in the foreground for converting cell to deep form.

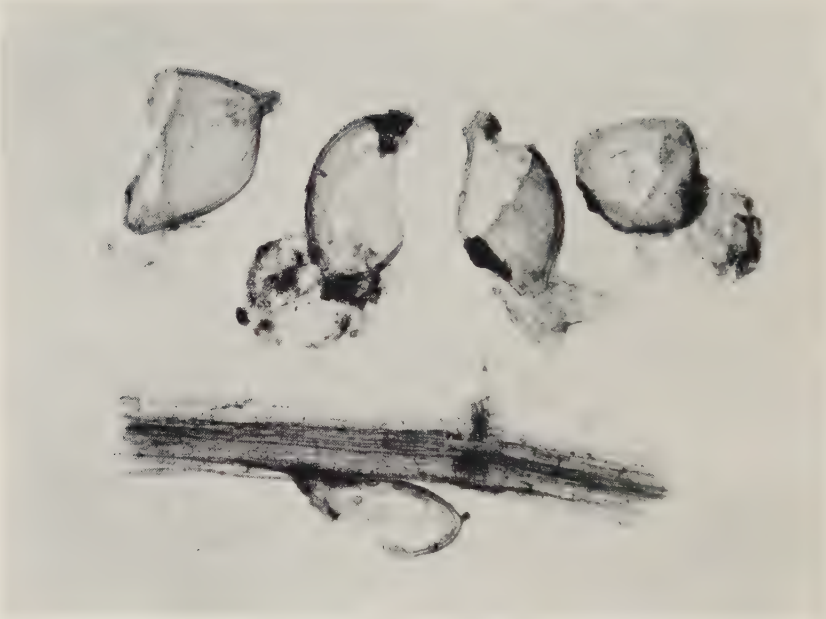

Fig. 4. Young adult females of *H. cruciferae* removed from rape plants in July, 1956. The damage shown is characteristic of that caused by Collembola.

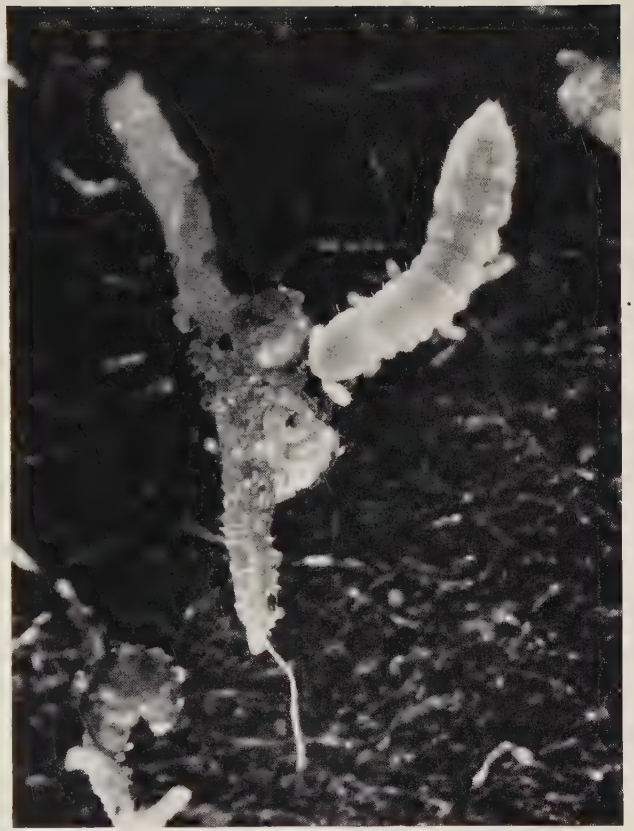

Fig. 5. *Onychiurus armatus* feeding on mature female *Heterodera cruciferae* (centre). A brown cyst of *H. cruciferae* which has been opened and the contents removed by *O. armatus* is also seen (bottom, left).

Fig. 6. The same culture as that illustrated in Fig. 5, photographed five hours later. Note the enlarged performations in the white *H. cruciferae* and the characteristic feeding attitude of *O. armatus*.

ably by means of extra-intestinal digestion, and WEBER, ZWILLENBERG and VAN DER LAAN (1952) reported an amoeboid organism engulfing and digesting larvae of *Heterodera rostochiensis* Wollenweber. This organism was later named *Theratromyxa weberi* Zwillenberg (ZWILLENBERG 1953 and VAN DER LAAN 1954).

Methods

In an attempt to find predators of *H. cruciferae,* soil samples collected from the top four inches of the culture plot in July and August, were elutriated in a 1 litre measuring cylinder and numerous Collembola and soil mites were recovered, together with a few Symphyla. By the 25th August no more Symphyla were being recovered and the most abundant soil animals were *Onychiurus armatus* (*s. lat.*) Tullb., *Folsomia* sp., *Hypogastrura* sp., *Isotoma viridis* Bourlet and other Collembola; various soil mites were also present in fair numbers. Later the meiofauna was extracted from the soil samples by means of a split-funnel extractor (MURPHY 1956) and most species recovered were cultured in selected groups in P. W. MURPHY's culture cells. Each group was supplied with a new cyst of *H. cruciferae* and the culture cells were irrigated daily.

Predators of Heterodera

By 31st August none of the cysts in the culture cells had been eaten and a soil sample was taken from a pot culture of *H. cruciferae* from which damaged cysts had been recovered. This culture contained *O. armatus* in large numbers as well as most of the other forms recovered from the plot. On 1st September one specimen of *O. armatus* was seen nibbling away part of the cuticle of a cyst which had recently turned brown. Feeding continued until a shallow depression had been made in the cyst contents, when the insect was removed for mounting.

About a week after its introduction into a culture cell containing a mature female *H. cruciferae,* an *Isotoma viridis* was seen feeding intermittently on the nematode. Damage to the nematode did not progress far and the contents had not completely disappeared. This springtail fed more intensively on cortical tissues of the root to which the cyst was attached.

In a mixed culture of Collembola consisting of *Folsomia* sp., *Hypogastrura,* sp., *Lepidocyrtus* sp., *Orchesella villosa* (Geoffroy) and *Iso-*

toma notabilis Schäffer, a fresh brown cyst of *H. cruciferae* was found to have been perforated and a juvenile *Hypogastrura* sp. (probably *denticulata* Bagnall) was seen feeding on the cyst contents. In the same culture cell, which did not then include the *Orchesella villosa* a fourth stage female *H. cruciferae* was completely consumed.

The *O. villosa* was placed in a separate culture cell where it was seen feeding on the cortices of root fragments. But a fortnight after its introduction it was found to have eaten away an area of cuticle of a cyst of *H. cruciferae,* which was turning from white to brown. However, feeding on the cyst was neither sustained nor extensive.

J. J. Hesling informed the authors that he had seen soil mites of the Suborder Mesostigmata feeding on egg-sacs of *Heterodera schachtii* var. *trifolii* Goffart and mites from this group were kept with both white and brown females of *H. cruciferae.* On one occasion a small part of the egg-sac of a cyst was found to have been removed, but there was no evidence that anything more than the gelatinous matrix had been eaten. On several occasions it was thought that mature female heteroderas placed with these mites turned a dark brown colour unusually quickly: on two occasions this happened within five hours of placing in the culture cell, whereas previously they had remained in the white condition in a similar storage cell for about a week. Similar cysts believed to have been affected by Mesostigmata were transferred to a separate culture cell and three to four weeks later were placed in a watch glass of Brussels-sprout root diffusate. There was a fair emergence of larvae, indicating that if the mites had in some way killed the adult nematodes there was not, at any rate, complete mortality of the eggs within the cysts.

Nematodes recorded as food of Onychiurus armatus

As *O. armatus* was by far the most voracious predator on *H. cruciferae,* a variety of nematodes were placed in culture cells each with 7 to 9 freshly caught Collembola of this species. Two large *Dorylaimus* and a *Mononchus* from soil were both partially eaten, one of the *Dorylaimus* within an hour of introduction into the cell: it was showing little activity when introduced and no evidence has been obtained that nematodes are ever attacked when actively moving. Brown (1954) however, reports actively moving nematodes (not identified) being smoothly and rapidly ingested by isotomid Collembola, as if sucked in by them; the *O. armatus* ate through the vermi-

form nematodes transversely and made no attempt to ingest entire eelworms.

Table I summarizes observations on the feeding of *O. armatus* on *H. cruciferae,* and provides data on the time elapsing before feeding commenced together with its extent and duration for each nematode. It is seen that living white females were preferred to brown cysts, although cysts were sometimes eaten if there were no live females available.

Attempts were made to feed *O. armatus* on white females of other species of *Heterodera.* Only *H. schachtii* and *H. schachtii* var. *trifolii* were then available (17th October), although rearing of suitable stages of other species is now being attempted. In the first culture, containing *H. schachtii* var. *trifolii,* there were 4 adult females, some with egg-sacs, and one fourth-stage larva. Within an hour of introduction, one young white female had been partially ingested and during the following night the fourth-stage female had been completely devoured. Three days later one specimen had turned brown, but was apparently undamaged and one mature white female remained unchanged. The remaining two specimens had been emptied of their body contents. No further change occurred.

The second culture contained three live females of *H. schachtii* with incompletely developed egg-sacs. One of these nematodes was punctured two days after its introduction, the head of another was eaten away at about the same time and some feeding from the base of egg-sacs was noticed. All three females had turned to brown cysts three days after their introduction and in this condition appeared to be immune to attack in this culture.

The usual behaviour of *O. armatus* when provided with fresh white *Heterodera* females on roots, was to spend a variable time examining the food with the antennae. Sometimes this would continue intermittently for several days. Finally, an insect would scrape a definite area of the nematode, employing a gentle abrasive action with the mandibles and persisting for up to an hour at a time. The antennae would be directed backwards, and other Collembola were apparently mildly deterred from interfering, by the predator pointing the tip of the abdomen and anal spines towards each intruder. The scraping period varied considerably and was usually interrupted more than once, but it is believed that the nematode cuticle was usually perforated within six to twelve hours of the commencement of scraping and sometimes within a much shorter period. Once the cuticle had been penetrated

TABLE I

Observations on *O. armatus* cultures provided with living females and cysts of *H. cruciferae*

Culture number (*O. armatus*)	Number of *O. armatus* in culture	Dates of introduction of *H. cruciferae*	Condition of *H. cruciferae*	Duration of feeding	Extent of damage to *H. cruciferae*	Other food present
1	?	25th August	Recently turned brown		None	None
2	9	31st August	Recently turned brown	1st Sept.-1st Sept.	Cuticle perforated; some contents removed	Root fragment
3	9	18th September	White	21st-25th Sept.	Contents removed	Rootlets and brown cyst
		18th September	Recently turned brown		None	Rootlets and white female
		25th September	White	27th Sept.-1st Oct.	Most of contents removed	Rootlets and brown cyst
		25th September	Recently turned brown		None	Rootlets and white female
4	9 reduced by death to 6	17th October	Small, white	17th-19th October	Completely ingested	Rootlets and brown cyst
		17th October	Recently turned brown	19th-22nd October	Part of cuticle only remained	Rootlets and white females
		18th October	White	18th-19th October	Part of cuticle only remained	Rootlets and brown cyst
		18th October	White	18th-19th October	Fragment of cuticle only remained	Rootlets and brown cyst
		22nd October	Dry, brown cyst		None	Root fragments
		26th October	White: turned brown in culture		None	Root and peat-moss fragments
		3rd December	White: turned brown in culture		None	Rootlets, peat-moss and cyst

other *O. armatus* would find the food supply and frequently as many as four or five individuals were seen simultaneously devouring a single nematode. During this time competition for the food was most acute and individuals were often seen attempting to repel each other, on these occasions by lunging at one another and by free use of the mandibles, when they bit antennae, head or thorax. This was sufficient to cause abrupt cessation of feeding or to drive off an intruder, although no permanent injuries were apparently ever sustained. During this period a single *Heterodera* female might be perforated in as many as three or four places, the holes being progressively enlarged as the insects ate deeper into the interior. Providing that suitable conditions were maintained in the culture, the predator always consumed the contents of the nematode whether in the white or brown state, except in the case of the single *H. schachtii* female which was only punctured. Young females with relatively thin, soft cuticles were sometimes completely devoured, but it appeared probable that the cuticle was less attractive than the contents and became even less so as it aged.

Estimation of damage by Collembola to a population of H. cruciferae

No attempt was made to obtain an accurate quantitative estimate of the amount of predation by Collembola in a population of *H. cruciferae* because the age of the *Heterodera* females seemed to have an effect on the amount of damage done to them and it is probable that some were completely ingested. However, a pot culture of *H. cruciferae* was examined in order to obtain a minimum estimate of springtail predation.

In October a cabbage root system infested by *H. cruciferae,* together with its adhering soil was weighed and then washed. The soil and washings were collected in a flask and all floating material was collected on a filter-paper for examination. The remaining soil was collected on a 100 mesh-to-the-inch sieve, dried and finally re-washed in order to recover floatable material. From the root system and the first and second floats, counts were made of the numbers of *O. armatus,* undamaged heteroderas, those damaged by unknown causes and those showing characteristic damage by Collembola. The results are given in Table II. The mean number of *O. armatus* per gram of soil and roots was 1.0 and of *H. cruciferae* 5.3.

In interpreting these results it must be borne in mind that only partially eaten heteroderas could be counted. Some damaged cysts still

TABLE II

Numbers of Collembola, normal and damaged *Heterodera cruciferae* individuals recovered from a cabbage root system (11th October)

(Fresh weight of root system and soil = 247 g)

	O. armatus	*H. cruciferae*			
		Undamaged	Damaged		
			Cause unknown	Damaged by Collembola	
				Numbers	Per cent, of total
H. cruciferae attached to cabbage root system		8	1	3	25.0
Float from moist soil	251	328	29	26	6.8
Float from dried residual soil	A few probable remains	706	60	55	6.7
Totals	251	1,042	90	84	
Grand Totals	251		1,216		6,9

contained eggs and the remainder were identified from fragments of cuticle of various sizes which were apparently sufficiently tough and resistant to survive the processes involved in their collection. However it is probable that during extraction many remnants of the more fragile white females were lost or damaged beyond recognition.

ACKNOWLEDGEMENTS

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ZUSAMMENFASSUNG

Nach einem Rückblick auf die Methoden der Kultivierung von Acariden, Collembolen und anderen Bodenorganismen werden zwei neue Kulturkammern beschrieben. Die kleinere besteht aus einem gläsernen Mikro-Immersions-Filter, der mit einer Spritze verbunden ist. Die Zelle wird mit Hilfe der Spritze befeuchtet. Sie ist besonders für Nahbeobachtungen bei Untersuchungen an Collembolen als Räuber von bodenbewohnenden und pflanzenparasitischen Nematoden, z.B. bei *H. cruciferae*, geeignet. Bei der grösseren Kammer wird anstelle des Immersionsfilters ein Gootsch-Filter-Schmelztiegel verwendet. Dieses Verfahren dient besonders zum Sammeln von lebendem Material aus Streu und Boden mit einem Extraktor nach Trichter-Art.

Zysten von *Heterodera cruciferae* aus Kulturen haben in der Kutikula häufig Löcher aufzuweisen. Die Collembolenarten *Onychiurus armatus, Isotoma viridis, Hypogastrura* sp. und *Orchesella villosa* frassen an ihnen. Milben der Unterordnung Mesostigmata töteten wahrscheinlich erwachsene Weibchen von *H. cruciferae* und fressen zu einem gewissen Grade an dem Eiersack. *O. armatus* war der wichtigste Räuber in den Kulturen; dieser frass auch an inaktiven Nematoden der Gattungen *Dorylaimus* und *Mononchus,* an Weibchen von *Heterodera schachtii* var. *trifolii* und bis zu einem gewissen Grade an *H. schachtii.* Auszählungen im Oktober zeigten, dass wenigstens 6,9 % der *H. cruciferae*-Zysten in Topfkultur von Collembolen geschädigt waren. Dabei war das Verhältnis der Zysten zu *O. armatus* wie 5 : 1.

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ERRATUM

Nematologica, 2: 7-15.

Page 10, fig. 2.

Nematodes per 10 milligrams of root *should read* Nematodes per 40 milligrams of root.

Nematologica II (1957): 215-227

BEZIEHUNGEN ZWISCHEN WIRT UND PARASIT IM INFEKTIONSVERLAUF VON *HETERODERA SCHACHTII* SCHMIDT IN KURZFRISTIGEN TOPFVERSUCHEN

II. HAUPT- UND NEBENWURZELBEFALL, GESCHLECHTSVERHÄLTNIS DER ADULTEN UND LAGERICHTUNG DER LARVEN [1])

VON

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DER BEFALL DER HAUPT- UND NEBENWURZELN VERSCHIEDENER WIRTSPFLANZEN DURCH H. SCHACHTII SCHM.

Wie aus früher wiedergegebenen Befunden (APEL & KÄMPFE, 1957) hervorgeht, besteht bei kurzfristigen Topfversuchen eine deutliche Abhängigkeit der Befallsstärke von *Heterodera schachtii* SCHM. von der Grösse des gebotenen Wurzelsystems. Für die Befallsverhältnisse der Haupt- und Nebenwurzeln ist daher zu erwarten, dass zunächst die Parasitenzahl in der Hauptwurzel absolut grösser sein wird, da im Anfang der Pflanzenentwicklung zunächst die Hauptwurzel stark überwiegt. Erst mit fortschreitender Entwicklung nehmen die Nebenwurzeln zu und übertreffen bald an Länge die Hauptwurzeln erheblich. Auch für die spätere Entwicklung ist ja der sog. Wurzelbart an befallenen Zuckerrüben geradezu charakteristisch für Nematodenbefall. Die Bevorzugung jüngerer Seitenwurzeln gegenüber der Hauptwurzel ist schon aus der leichteren, mechanischen Invasionsmöglichkeit für die Larven verständlich.

Für alle vier hier benutzten Wirtspflanzen (Zuckerrüben, Rübsen, Senf und Raps) weist der absolute Befall durch *Heterodera schachtii* SCHM. insofern völlig übereinstimmende Verhältnisse auf, als mit dem Ansteigen der Nebenwurzellänge auch die Zahl der in diesen Wurzeln befindlichen Parasiten gleichsinnig ansteigt. Unmittelbar nach Überwiegen der Nebenwurzellängen über die der Hauptwurzeln folgt regelmässig der Anstieg der Befallskurve für Nebenwurzeln über die der Hauptwurzel. Lediglich für Rübsen mit ihrem relativ schwachen Allgemeinwachstum der Wurzeln bleibt die Nebenwurzellänge ständig

1) Eingegangen: 9. September 1956.

unter der der Hauptwurzel. Damit erreicht auch der absolute Befall der Nebenwurzeln im gesamten Versuchsverlauf nie die Höhe wie in der Hauptwurzel.

Da sich die Verhältnisse für die einzelnen geprüften Wirtspflanzen, abgesehen von der eben erwähnten nur graduellen Abweichung bei Rübsen, sehr ähneln und nur zeitliche Verschiebungen aufweisen,

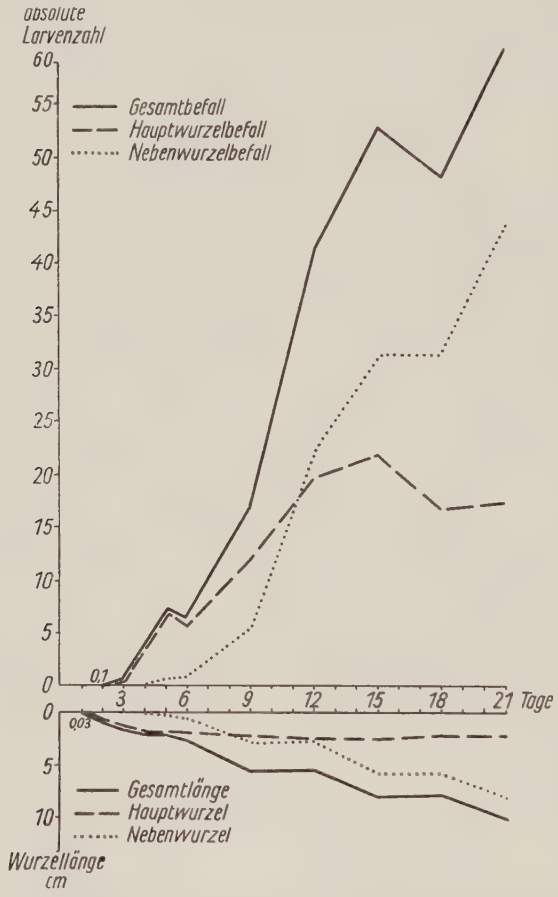

Abb. 1: Absoluter Befall mit *Heterodera schachtii* SCHM. in Haupt- und Nebenwurzeln für Raps als Wirtspflanze.

die von der Stärke und Schnelligkeit des Wurzelwachstums abhängen, kann auf die Wiedergabe von Kurven für jede Wirtspflanze verzichtet werden. Als Beispiel sind lediglich die Verhältnisse für Raps in Abb. 1 dargestellt.

Es lässt sich — wiederum mit Ausnahme von Rübsen — feststellen, dass der Gipfel im absoluten Befall des Gesamtwurzelsystems immer dann auftritt, wenn die Nebenwurzeln ein Maximum an Befall erreicht haben und wenn auch in der Hauptwurzel noch verhältnismässig viel Parasiten vorhanden sind. Für Rübsen liegen die Verhältnisse insofern anders, als das Befallsmaximum schon sehr früh (am 9. Versuchstage) eintritt, nämlich zu einer Zeit, zu der praktisch noch keine Nebenwurzeln gebildet sind. Dieses Maximum wird also fast ausschliesslich von den Larven in der Hauptwurzel aufgebaut.

Für alle Versuchspflanzen zutreffend ist weiter der Befund, dass mit Ansteigen des Nebenwurzelanteiles und mit dem damit verbundenen Einwandern von Larven II in diese die Zahl der Larven II in der Hauptwurzel auffallend zurückgeht. Darin kann ein deutlicher Beweis für die grosse Attraktivwirkung der stark wachsenden, jüngeren Nebenwurzeln gegenüber der älteren und weniger stark wachsenden Hauptwurzel gesehen werden. Die Befunde stehen in Einklang mit den Ergebnissen, die WIESER (1955) kürzlich hinsichtlich der Anlockwirkung von Tomatenwurzeln auf *Meloidogyne hapla* CHITWOOD gefunden hat. Eine nähere Analyse der Verteilung der einzelnen Stadien des Parasiten im Wurzelsystem bei kürzeren Kontrollzeiten unter Zugrundelegung der Befunde von WIESER wäre recht interessant.

Neben der Kenntnis der absoluten Befallszahlen in Haupt- und Nebenwurzeln lässt die Betrachtung der Befallsdichte, bezogen auf 10 cm Wurzellänge, weitere Aufschlüsse und Vergleiche erwarten. In Abb. 2 ist jeweils die Befallsdichte und die Länge der Haupt- und Nebenwurzeln dargestellt. Dabei zeigen sowohl die Haupt- als auch die Nebenwurzellängen — wieder mit Ausnahme der Nebenwurzeln bei Rübsen — etwa übereinstimmende Tendenz und Lage ihrer Kurven. Demgegenüber weichen die Befallsdichten stärker voneinander ab. Die Ursache kann also weniger in einem unterschiedlichen Wachstum des Wurzelsystems, oder — anders ausgedrückt — in der unterschiedlich grossen Wurzelmasse gesehen werden, sondern dürfte vielmehr auf qualitative Unterschiede, durch die sich die Wurzeln der verschiedenen Wirtspflanzen auszeichnen, zurückzuführen sein. Die Differenzen können sowohl auf der Ebene der chemisch-physiologischen Beziehungen zwischen Wurzel und Parasit bei der Anlockung liegen oder durch die verschiedenen mechanischen Eigenschaften der Wurzelepidermen der Wirte mit bedingt, als auch durch die Grösse des notwendigen Minimallebensraumes für den Parasiten in der Wurzel beeinflusst werden. Dieser hängt wiederum von einem Komplex ver-

schiedener Wirt-Parasit-Beziehungen ab. Auf das Bestehen einer kritischen Phase in der Entwicklung des Wurmes als ein Faktor soll später noch eingegangen werden.

Der Vergleich der Befallsdichte in Haupt- und Nebenwurzel zeigt nun, für jede Wirtspflanze einzeln betrachtet, einen in grossen Zügen ähnlichen Verlauf, wobei von einzelnen Abweichungen abgesehen werden soll. Es lässt sich vor allem trotz z.T. unregelmässigem Kurvenverlauf feststellen, dass beispielsweise die Maxima der Befallsdichte in

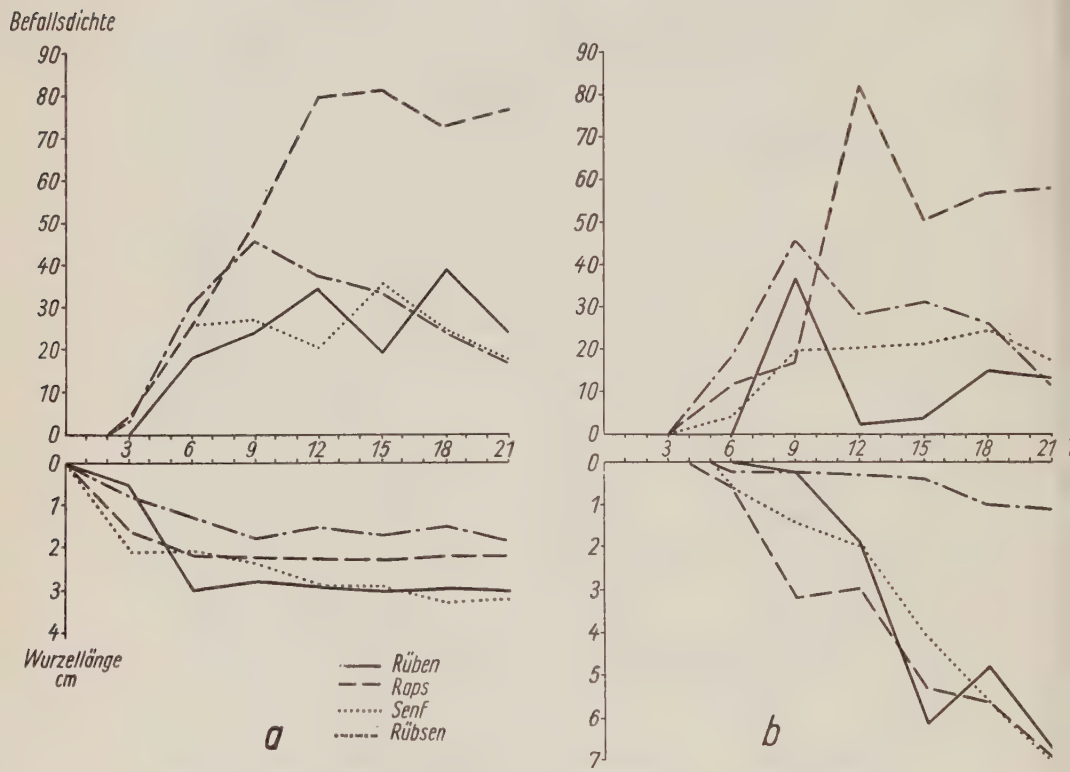

Abb. 2: Befallsdichte in Haupt- und Nebenwurzeln für Rüben, Raps, Senf und Rübsen in Hauptwurzel (a) und Nebenwurzeln (b).

Haupt- und Nebenwurzeln für die einzelnen Wirtspflanzen jeweils in einem Niveau zu finden sind. Das deutet wiederum darauf hin, dass eine für jede Wirtspflanze möglicherweise spezifische und normalerweise nicht überschreitbare Besiedlungsdichte gegeben ist. Dies gilt selbstverständlich vorläufig nur für die hier gewählte Versuchsdauer. Auch hier sind Untersuchungen im Freiland mit längerer Versuchs-

dauer erwünscht und vorgesehen. Trotz starkem Ansteigen der absoluten Befallszahlen besonders in den Nebenwurzeln wird eine grösste Dichte niemals überschritten. Dabei dürfte ein regulierender Einfluss durch das fortschreitende Wurzelwachstum, das immer wieder eine steigende Befallsdichte aufzulockern vermag, nicht zu verkennen sein. Wurzelwachstum und Einwanderung scheinen also bis zu einem gewissen Masse korreliert zu sein, wobei die Verknüpfung beider Faktoren vor allem das Überschreiten einer Norm nach oben, weniger nach unten, zu erreichen sucht. Eine interessante Parallele stellen die jüngsten Untersuchungen von JONES (1956) dar, in deren Rahmen der Populationszuwachs nach Anbau verschiedener Pflanzenarten für den Rübennematoden geprüft wurde. Dabei zeigte es sich, dass trotz verschiedener Ausgangsverseuchung eine bestimmte Höhe der Endpopulation nicht überschritten wird. Weiter konnte nachgewiesen werden, dass Cruciferen als Wirtspflanzen einen stärkeren Populationszuwachs bedingen als Chenopodiaceen, wenn die Ausgangspopulation relativ gering ist. Alle Befunde deuten auf ein kompliziertes System zur Erzielung eines ausgewogenen Verhältnisses des Parasiten zu seiner Wirtspflanze hin. Ein solches System stellt in unbeeinflusster Umwelt und über längere Zeiträume betrachtet sicher optimale Bedingungen für den angreifenden Parasiten wie auch für die dem Befall ausgelieferte Wirtspflanze dar.

Inwieweit ein weiterer Faktor die Dichte des Befalls in der Wurzel beeinflussen könnte, soll im folgenden angedeutet werden. Ein Vergleich der absoluten Zahlen für die eingewanderten Larven II und für die sich in der Wurzel entwickelnden Larven III in Haupt- und Nebenwurzeln deutet auf einige Unterschiede hin. Dabei sind die Larven II, die nach dem 15. Tage eingewandert sind, nicht mit berücksichtigt worden, weil etwa 5 bis 6 Tage für die Entwicklung der Larve III aus der soeben eingewanderten Larve II veranschlagt werden müssen.

Die folgende Übersicht gibt den Anteil der Larven III in % von der Zahl der eingewanderten Larven II für Haupt- und Nebenwurzeln getrennt wieder (Tabelle I).

In allen 3 genannten Fällen ist also der höhere Anteil an Larven III, bezogen auf die festgestellte Zahl der eingewanderten Larven, in den Nebenwurzeln deutlich und statistisch gesichert. Für Zuckerrüben ist die Berechnung wegen des zu späten Auftretens der Larven III in den Nebenwurzeln nicht möglich. Die Betrachtung der wenigen vorhandenen Zahlen deutet aber auch hier in die oben angegebene Richtung.

Tabelle I

Rübsen

	Larven II	Larven III	Larven III in %
Hauptwurzel	518	139	26,8 ± 1,95
Nebenwurzeln	86	42	48,8 ± 5,42
		D = 22,0 ± 5,66 %	

Raps

Hauptwurzel	997	556	55,8 ± 1,57
Nebenwurzeln	1200	1096	91,25 ± 0,81
		D = 35, 45 ± 1,77 %	

Senf

Hauptwurzel	614	160	26,1 ± 1,76
Nebenwurzeln	270	266	98,5 ± 0,74
		D = 72,4 ± 1,91 %	

Hieraus lässt sich bei aller zu Gebote stehenden Vorsicht als allgemeine Tendenz annehmen, dass beim Übergang von einem Stadium in das nächste Häutungsstadium sicher Verluste eintreten. Dies ist z.B. auch bei Arthropoden der Fall, bei denen die Häutungen besonders gefährdete Phasen der Entwicklung darstellen. Dass dies in hohem Masse ebenfalls für den Übergang von Larve II zu Larve III zutreffen mag, wird vielleicht durch die Überlegung gerechtfertigt, dass es für die frei im Erdboden wandernde Larve nach ihrem Eindringen in die Wurzel mit ihren völlig anderen Lebensbedingungen und mit dem Einsetzen der aktiven Nahrungsaufnahme einer besonders tiefgreifenden Umstellung physiologischer Art bedarf und dass ungünstige Verhältnisse, falls sie in der Wurzel herrschen, den zu vollziehenden Übergang in das nunmehr voll parasitische Larvenstadium bei der Häutung verhindern können.

Ein extremer Fall, der in die gleiche Richtung deutet, ist von Doncaster (1953) für *Heterodera rostochiensis* Wollenweber beschrieben worden. Dieser Nematode zeigte bei Befall von *Solanum nigrum* starke Entwicklungsverzögerungen, Degenerationserscheinungen und vor allem grosse Ausfälle beim Übergang zur Larve III.

Es ist in diesem Zusammenhang bezeichnend, dass in Rapswurzeln, die den höchsten absoluten und relativen Befall aufweisen, die Zahl der Larven III der der eingewanderten Larven II am nächsten kommt. Der physiologische Sprung zur Weiterentwicklung in der Wurzel wird offenbar von sehr vielen eingewanderten Larven mit Erfolg vollzogen,

was wiederum auf die günstige Situation für Befall durch *Heterodera schachtii* SCHM. gerade in Raps als Wirtspflanze hindeutet.

Welche Gründe es sind, die einen geringeren Prozentsatz von Larven III in den Hauptwurzeln aller Versuchspflanzen im Vergleich zu ihren Nebenwurzeln hervorrufen, ist noch unbekannt. Es ist nur soviel anzunehmen, dass in den Hauptwurzeln schlechtere Bedingungen herrschen müssen als in den Nebenwurzeln. Ob dadurch eine gewisse Regulierung der Befallsdichte in der Wurzel erreicht werden kann oder soll, ist fraglich, wäre aber zumindest denkbar. Wir hatten ja vorhin auf die etwa gleiche Lage der Dichtemaxima im Haupt- und Nebenwurzelsystem für jede Wirtspflanze hingewiesen. Unter der Voraussetzung, dass der Anteil der Larven III in den Hauptwurzeln ebenso hoch läge wie in den Nebenwurzeln, müsste zwangsläufig in den Hauptwurzeln eine höhere Besiedlungsdichte eintreten, was aber auf Grund unserer Befunde nicht der Fall ist.

Unser Wissen um die speziellen Beziehungen zwischen Wirt und Parasit hinsichtlich des Infektionsverlaufes sind noch viel zu lückenhafte, um über Vermutungen hinauszukommen. Auch die hier angeführten Befunde sind eine viel zu schmale Grundlage, als dass sichere Schlüsse möglich wären. Die angedeuteten Überlegungen schienen jedoch wert, einmal diskutiert zu werden. Es ist nicht ausgeschlossen, dass auch in der Entwicklung von *Heterodera schachtii* SCHM. eine oder mehrere kritische Phasen bestehen, deren Überwindung für den Befall massgebend ist. Eine solche stellt sicher die Auffindung und das Eindringen in die Wurzel dar; eine zweite kritische Phase, die in der Wurzel selbst abläuft, könnte im Bereich des Möglichen liegen.

GESCHLECHTSVERHÄLTNIS DER ADULTEN UND LAGEBEZIEHUNGEN DER LARVEN II ZUR WURZELSPITZE

Über das Geschlechtsverhältnis des Rübenälchens liegt schon eine Anzahl von Untersuchungen vor, deren Ergebnis z.T. recht unterschiedlich ist (vergl. STRUBELL, 1888; MOLZ, 1920 u. 1927, v. SENGBUSCH, 1927). In jüngster Zeit hat ELLENBY (1954) die Frage für das Kartoffelälchen erneut aufgegriffen und einige bemerkenswerte Gedanken geäussert. Es ist seit längerem bekannt, dass sowohl unterschiedliche abiotische Faktoren als auch die Eigenart der betreffenden Wirtspflanze Einfluss auf das Geschlechtsverhältnis nehmen.

Die folgende Übersicht (Tabelle II) zeigt das Ergebnis unserer Auszählungen am 21. Tage, also zu einem Zeitpunkt, an dem kaum mit bereits begonnenem Auswandern der Männchen aus den Wurzeln

Tabelle II

	♂	♀	D in %	♀ : ♂	durchschnittl. Besiedlungsdichte Larven/10 cm
Rüben	44	132			
	25,0 ± 3,23	75,0 ± 3,23	50,0 ± 4,53	1 : 0,33	11,5
Rübsen	26	11			
	70,3 ± 7,75	29,7 ± 7,75	40,6 ± 10,90	1 : 2,36	21,6
Senf	171	36			
	82,6 ± 2,64	17,4 ± 2,64	65,2 ± 3,74	1 : 4,75	13,8
Raps	507	200			
	71,4 ± 1,71	28,6 ± 1,71	42,8 ± 2,41	1 : 2,54	35,1

zu rechnen ist. Es wurden alle Stadien als Männchen bzw. Weibchen gewertet, die durch ihre Form sicher einzuordnen waren, also auch Tiere als Männchen gezählt, die noch in der letzten Larvenhaut aufgerollt lagen. Raski (1950) gibt als Zeitpunkt, an dem die meisten Männchen die Wurzeln verlassen, 20 Tage bei einer Temperatur von 66.8° F (= ca. 19° C) an. Da die Einwanderung der Masse junger Larven in unserem Falle erst ab 3. Versuchstag stattfindet, ist also damit zu rechnen, dass die Männchen bei der Kontrolle am 21. Tag weitgehend erfasst worden sind.

Die Entwicklung der Weibchen hat der gleiche Autor mit 16-17 Tagen, das erste Auftreten von Eiern in den Gonaden mit 23-24 Tagen veranschlagt. Wenn auch besonders die erste Zeitangabe nach unserer Erfahrung etwas zu knapp bemessen scheint, so ist es aber doch möglich, am 21. Tage sicher die Tiere zu erkennen, die im Begriff sind, sich zu adulten Weibchen zu entwickeln. Die Larven IV, bei denen dies u.U. auch schon möglich wäre, sind von uns nicht berücksichtigt worden.

Die Zusammenstellung zeigt, dass lediglich in Rübenwurzeln nach 21 Tagen mehr Weibchen als Männchen vorhanden sind. In allen übrigen Wirtspflanzen überwiegen die Männchen, am stärksten bei Senf. Diese Befunde lassen weitgehende Übereinstimmung mit den Ergebnissen von Molz (1927) erkennen. Allerdings fand Molz für Zuckerrüben kein Überwiegen der Weibchen, aber auch in seinen Versuchen wies diese Wirtspflanze Werte auf, die sich am stärksten einem Verhältnis von 1 : 1 näherten. Er fand ein solches von 1 : 1,54. Auch bei ihm liegt Senf am ungünstigsten mit 1: 4,49.

In der oben bereits erwähnten Arbeit von Ellenby (1954) wird festgestellt, das für Kartoffelnematoden mit dem Ansteigen der Befalls-

intensität ein Ansteigen des Männchenanteils verbunden ist und weiter das Geschlechtsverhältnis in der Hauptwurzel ausgeglichener ist als in den Nebenwurzeln, da in diesen die Männchen wiederum stärker überwiegen. Die in unserer Tabelle angeführten mittleren Befallsdichten für jede Wirtspflanze deuten insofern in die gleiche Richtung, als bei Rüben die geringste Befallsdichte mit dem günstigsten Weibchen: Männchen-Verhältnis verbunden ist. Die übrigen Werte lassen jedoch keine strenge Abhängigkeit erkennen. Es ist allerdings zu berücksichtigen, dass der Einfluss der Befallsdichte auf das Geschlechtsverhältnis weitgehend von der sonstigen, insbesondere physiologischen Eigenart der betreffenden Wirtspflanze überdeckt sein kann.

Die von Ellenby festgestellte Differenz in Haupt- und Nebenwurzeln konnte von uns in anderen Versuchen für Raps und Rübenälchen ebenfalls gefunden werden. Ellenby erklärt die Unterschiede im Geschlechtsverhältnis mit einem Modus der Determination des Geschlechts, der unter dem Einfluss der Umwelt („under environmental influence") steht; er lehnt die Möglichkeit einer unterschiedlichen Absterberate der zukünftigen Männchen und Weibchen vor dem Einwandern oder später in der Wurzel ab. Unser Material reicht nicht aus, um zu diesen Fragen definitiv Stellung zu nehmen. Im Falle der Bestätigung von Ellenbys Meinung wäre die Verfolgung der Geschlechtsbestimmung bei den in Frage stehenden Nematoden sehr interessant, zumal ihre Aufklärung von allgemeinerer Bedeutung werden könnte. Es sei in diesem Zusammenhang nur an die von Hartmann und Huth (1936) an *Ophryotrocha puerilis* Clap.-Metschn. gewonnenen Ergebnisse erinnert.

Cobb, Steiner u. Christie (1927) konnten übrigens auch bei dem in Heuschrecken parasitierende Nematoden *Mermis subnigrescens* Cobb eine umweltbedingte Steuerung des Geschlechtsverhältnisses feststellen. Stark parasitierte Wirte enthielten praktisch nur Männchen, während schwach befallene Wirte fast ausschliesslich Weibchen aufwiesen.

Auf Grund der bis heute vorliegenden Befunde kann allgemein festgestellt werden, dass

1. dem Nematoden günstige Bedingungen die Weibchenentwicklung fördern. Unter günstigen Bedingungen wäre auch eine geringe Besiedlungsdichte des Wurzelsystems zu verstehen;
2. verschiedene Wirtspflanzen offenbar auch ein verschiedenes Geschlechtsverhältnis bedingen können;

3. die bevorzugtesten Wirtspflanzen nicht unbedingt auch das günstigste Geschlechtsverhältnis aufweisen müssen, da sich offenbar Besiedlungsdichte bzw. Minimallebensraum und sonstige Eigenschaften der Pflanzenart als Wirt in noch unbekannter Weise überlagern.

Schliesslich ist im Rahmen unserer Untersuchungen die Frage geprüft worden, welche Richtung die eingewanderten Larven II in der Wurzel bevorzugen. Franklin (1951) und auch Raski (1950) geben an, dass die Larven parallel zum Zentralzylinder der Wurzel mit der Schwanzspitze zur Wurzelspitze liegend zur Ruhe kommen. Die hier angeführten Werte beziehen sich nur auf die Larven, deren Lage in der Wurzel sicher bestimmt werden konnte. Es wurden folgende Verhältnisse aufgefunden (Tabelle III):

Tabelle III

	Hauptwurzel Kopf zur W.-Basis %	Kopf zur W.-Spitze	n	Nebenwurzel Kopf zur W.-Basis %	Kopf zur W.-Spitze	n
Zuckerrüben	52,8 ± 1,44	47,2 ± 1,44	1225	70,1 ± 2,29	29,9 ± 2,29	394
	$D \pm m_D = 5{,}6 \pm 1{,}98\ \%$			$D \pm m_D = 40{,}2 \pm 3{,}25\ \%$		
	$3\ m_D > D$			$3\ m_D < D$		
Rübsen	59,0 ± 1,86	41,0 ± 1,86	705	83,2 ± 2,74	16,8 ± 2,74	184
	$D \pm m_D = 18{,}0 \pm 2{,}55\ \%$			$D \pm m_D = 66{,}4 \pm 3{,}96\ \%$		
	$3\ m_D < D$			$3\ m_D < D$		
Senf	57,2 ± 1,56	42,8 ± 1,56	1020	67,2 ± 1,47	32,8 ± 1,47	1010
	$D \pm m_D = 14{,}4 \pm 2{,}26\ \%$			$D \pm m_D = 34{,}4 \pm 2{,}12\ \%$		
	$3\ m_D < D$			$3\ m_D < D$		
Raps	52,9 ± 0,99	47,1 ± 0,99	2526	67,8 ± 0,80	32,2 ± 0,80	3421
	$D \pm m_D = 5{,}8 \pm 1{,}41\ \%$			$D \pm m_D = 35{,}6 \pm 1{,}13\ \%$		
	$3\ m_D < D$			$3\ m_D < D$		

Damit kann als bestätigt gelten, dass die eingewanderten Larven überwiegend die von Franklin und Raski angegebene Lage in den Wurzeln mit dem Kopf zur Wurzelbasis einnehmen, und zwar für alle hier geprüften Versuchspflanzen mit Ausnahme der Tiere in den Hauptwurzeln von Zuckerrüben. Hier ist das leichte Überwiegen der Larven, die mit dem Kopf nach oben zeigen, nicht statistisch gesichert. Die etwa bestehende Gleichheit beider Lagemöglichkeiten liegt jedoch im Bereich einer Zufälligkeit.

Welche Reize es sind, die die Larven veranlassen, eine bestimmte Lage in der Wurzel einzunehmen und welche Vorteile sich aus dieser bevorzugten Lage für die Larven ergeben könnten, ist noch unbekannt.

Wenn man die Befunde von Němec (1910) mit heranzieht, der schreibt, dass die Larven nach Eindringen in die Columella der Wurzel mit dem Kopf zur Wurzelbasis liegen, bei seitlichem Eindringen in die Wurzel jedoch mit dem Kopf zur Wurzelspitze gerichtet sind, wären die Befunde verständlich. Es liesse sich damit auch eine Erklärungsmöglichkeit für die auffällige Feststellung finden, dass in den Hauptwurzeln die Bevorzugung der Lage „Kopf zur Wurzelbasis" nicht so ausgeprägt ist wie in den Nebenwurzeln. Bezogen auf die Wurzelmasse ist der Anteil des für die Einwanderung günstigen Wurzelspitzenbereiches bei den Nebenwurzeln zweifellos grösser als bei der Hauptwurzel. Davon kann man sich bei jeder Betrachtung eines Wurzelsystems leicht überzeugen. Damit bestehen auch in den Nebenwurzeln grössere Möglichkeiten für die Larven, im Bereich der Wurzelspitze einzudringen, woraus sich ihre Lage mit dem Schwanz zur Wurzelspitze in der von uns festgestellten überwiegenden Mehrzahl der Fälle erklären liesse. Schliesslich ist es nicht ausgeschlossen, dass auch rein mechanische Faktoren noch eine Rolle spielen. Es konnte nämlich wiederholt beobachtet werden, dass eingewanderte Larven hakenförmig gekrümmt im Wurzelgewebe liegen. Solche Larven wurden in die Rubrik eingeordnet, die ihrer Kopfrichtung entsprach. Diese Lage der Larven deutet aber auf eine im Wurzelgewebe durchgeführte Wendung um 180° hin. Eine solche Kehrtwendung als sekundär von der Larve durchgeführte Korrektur ihrer Lage ist aber sicher in den im allgemeinen jüngeren und damit im Gewebe lockereren Seitenwurzeln leichter durchführbar als in der Hauptwurzel. Damit liesse sich auch der Befund gut in Einklang bringen, dass gerade bei Zuckerrüben keine statistisch gesicherte Bevorzugung einer Lagerichtung in der Hauptwurzel zu finden war. Die Hauptwurzel der Zuckerrüben wird schon frühzeitig dicker als die der übrigen untersuchten Pflanzen, was mit der späteren Entwicklung eines wohlausgebildeten Rübenkörpers zusammenhängt.

Ob auch rein ernährungstechnisch die Lage der Riesenzellen oberhalb des Kopfes des Parasiten, d.h. also in Richtung Wurzelbasis, für deren Herausbildung und Versorgung günstiger ist, kann hier nur als Frage angedeutet werden.

Die hier dargestellten Ergebnisse und erörterten Fragen stellen einen Teil von Untersuchungen über die Autökologie einiger pflanzenparasitischer Nematoden dar, die im hiesigen Institut durchgeführt werden. Es ist bewusst auf die Problematik einiger Fragen eingegangen worden, auch wenn Lösungen noch nicht in jedem Falle greifbar sind. Die

Verfolgung der in kurzfristigen Topfversuchen durchgeführten Versuche unter Freilandbedingungen sind in Aussicht genommen, sobald die Möglichkeit dazu besteht.

SUMMARY

The invasion of beet eelworm larvae into the main and lateral rootlets of host plants was studied. The distribution of larvae depends on the length of root in proportion to its volume. The peak of larval density in the whole root system is determined by reinforced invasion of the lateral roots. After the peak, new invasions succeed only in the lateral roots. Comparison of larval density in main and lateral rootlets of each host plant shows certain similarities in the maximum level of larval density. For each host plant there is a population density which normally is not exceeded. Factors regulating larval density in the roots are discussed. In the main roots, less larvae are able to reach stage III than in the lateral roots. The occurrence of one or more critical phases in the development of the beet eelworm are presumed. The sex ratio on the twenty first day appeared to be influenced by the kind of host plant, usually in the direction of surplus males. The influence of larval density on sex ratio may be overshadowed by the effect of different host plants. Except in the main root of sugar beet, the orientation of the larva within the root is usually with the tail towards the root tip, and this orientation is more strongly marked in lateral roots than in main roots.

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Nematologica II (1957) : 228-237

SYMPTOMS OF SUSCEPTIBILITY AND RESISTANCE IN SEEDLINGS OF RED CLOVER ATTACKED BY THE STEM EELWORM *DITYLENCHUS DIPSACI* (KÜHN) FILIPJEV [1])

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In certain regions of the Netherlands heavy damage to red clover (*Trifolium pratense* L.) is caused by the stem eelworm (*Ditylenchus dipsaci* (Kühn) Filipjev). The disease spreads rapidly during the year after sowing, resulting in a low yield of the second cut. In breeding for resistance, FRANDSEN (1951) and BINGEFORS (1952 and 1957) distinguished two groups of plants, those with swellings, which were called susceptible and those without which were presumed to be resistant. A detailed description of symptoms in resistant plants was not given, so that it may be assumed that they appeared normal.

SEINHORST (1956) inoculated seedlings of red clover with stem eelworms derived from rye (*Secale cereale* L.), onions (*Allium cepa* L.), mangolds (*Beta vulgaris* L.), potatoes (*Solanum tuberosum* L.) and red clover. GOODEY (1941 and 1950) inoculated with stem eelworms from oats (*Avena sativa* L.). Eelworms from hosts other than red clover caused brown spots on the hypocotyl, the cotyledons and their petioles. The plants did not exhibit swellings such as occur in red clover inoculated with the red clover race of the stem eelworm. SEINHORST reported that growth was sometimes inhibited and that this led to gall-like swellings near the growing point of the plant, but the cotyledon petioles were never swollen. GOODEY (1950) and SEINHORST (1956) established that the eelworms were unable to multiply in the plants with brown spots. Red clover can therefore be considered resistant to eelworms from other hosts. By inoculating the variety Merkur with stem eelworms from red clover, SEINHORST obtained, in addition to swollen plants, a large number of plants showing the same symptoms as those obtained by inoculating with eelworms from rye, etc. In these plants no multiplication of eelworms could be established. Thus, within the variety Merkur there are plants resistant to the red clover stem eelworm which can be recognized by brown spots.

1) Received for publication: March 16, 1957.

In this paper the results of a detailed study of symptoms associated with resistance and susceptibility in red clover are considered.

Methods and materials. Susceptible and resistant varieties and families of red clover were inoculated with eelworms derived from red clover. Eelworms were isolated from diseased plants by the method of SEINHORST (1950). Plants were raised and inoculated, with some alterations, by FRANDSEN's (1951) modification of BINGEFORS (1952 and 1957) method (DIJKSTRA 1956). Plants were raised on filter papers and inoculated between the filter papers as soon as the cotyledons had emerged from the seed coats.

Symptoms. When inoculated seedlings were compared with controls, the two groups of plants were clearly distinguishable, a week after inoculation, in the varieties Merkur and Offer which produced 60 % and 96 % of susceptible plants respectively. In the non-inoculated plants the first true leaves developed normally. Nearly all inoculated plants showed some inhibition of growth but this could be observed only when a sufficient number of active eelworms (some twenty to thirty per plant) were used.

Inoculated seedlings of Merkur could be divided first into swollen (fig. 1A) and non-swollen plants. The former were galled especially at the growing point, at the base of the cotyledon petioles and at the top of the hypocotyl. The swollen tissue was often a little lighter in colour than healthy tissue due to larger intercellular air spaces. The non-swollen plants in their turn could be divided into plants with inhibited growth (Fig. 1 B.C.) and healthy-looking plants. The latter occurred only in small numbers in our experiments. Plants with inhibited growth often showed brown, necrotic stripes which looked irregular under low magnification because they are intersected by areas which were not brown. The surface of affected parts was uneven with a more or less corroded appearance. The brown stripes occurred mainly on the upper part of the hypocotyl and on the lower part of the cotyledon. Heavily inoculated groups of plants contained many individuals with dark brown stripes while in lightly inoculated plants the stripes were lighter in colour. Heavily attacked plants were entirely brown around the growing point and were soon killed. Besides brown stripes, distortions of the cotyledons and their stalks often occurred.

Other red clover varieties and families showed the same range of symptoms as in Merkur, except that the proportions of the various groups were often quite different.

The classification of the plants in the groups mentioned above was usually easy, but sometimes difficulties arose:

1. The degree of swelling varied greatly within and between different populations. In Table 1, the frequency of the various groups in the susceptible variety Gendringen red clover and the fairly resistant variety Merkur are shown. In the susceptible variety Gendringen red clover many more strongly swollen plants occurred than in Merkur.

TABLE 1

Frequency of groups of plants with different symptoms in two clover varieties

Variety of red clover	Total number of plants	Non-swollen plants with stunted growth	Galled plants			Healthy-looking plants	Doubtful	Slightly / Greatly swollen
			slightly swollen	greatly swollen	total			
Gendringen	157	4%	1%	94%	95%	0%	1%	0.01
Merkur	160	31%	29%	31%	60%	5%	4%	0.94

Fig. 3 gives the percentage of galled plants in Roosendaal, Merkur and various families of red clover arranged in decreasing order (upper part of graph) and the percentage of greatly swollen plants among those galled (lower part of the graph).

From table 1 and Fig. 3 there appears to be a positive correlation between the percentage of galled plants in a population and the degree of swelling of the separate plants.

2. In plants two to three weeks old some individuals showed the typical brown stripes caused by eelworm attack on the upper side of the hypocotyl while the petioles of the first leaf had slight but typical swellings.

3. One week after inoculation plants were sometimes found with a brown discoloration near the growing point which was also slightly swollen at the same place. SEINHORST (1956) observed the same symptoms in his experiments. It was assumed that these were resistant plants, into the terminal buds of which many eelworms had penetrated and caused swellings. After the second week this swelling had not extended. In susceptible plants on the other hand the swollen parts

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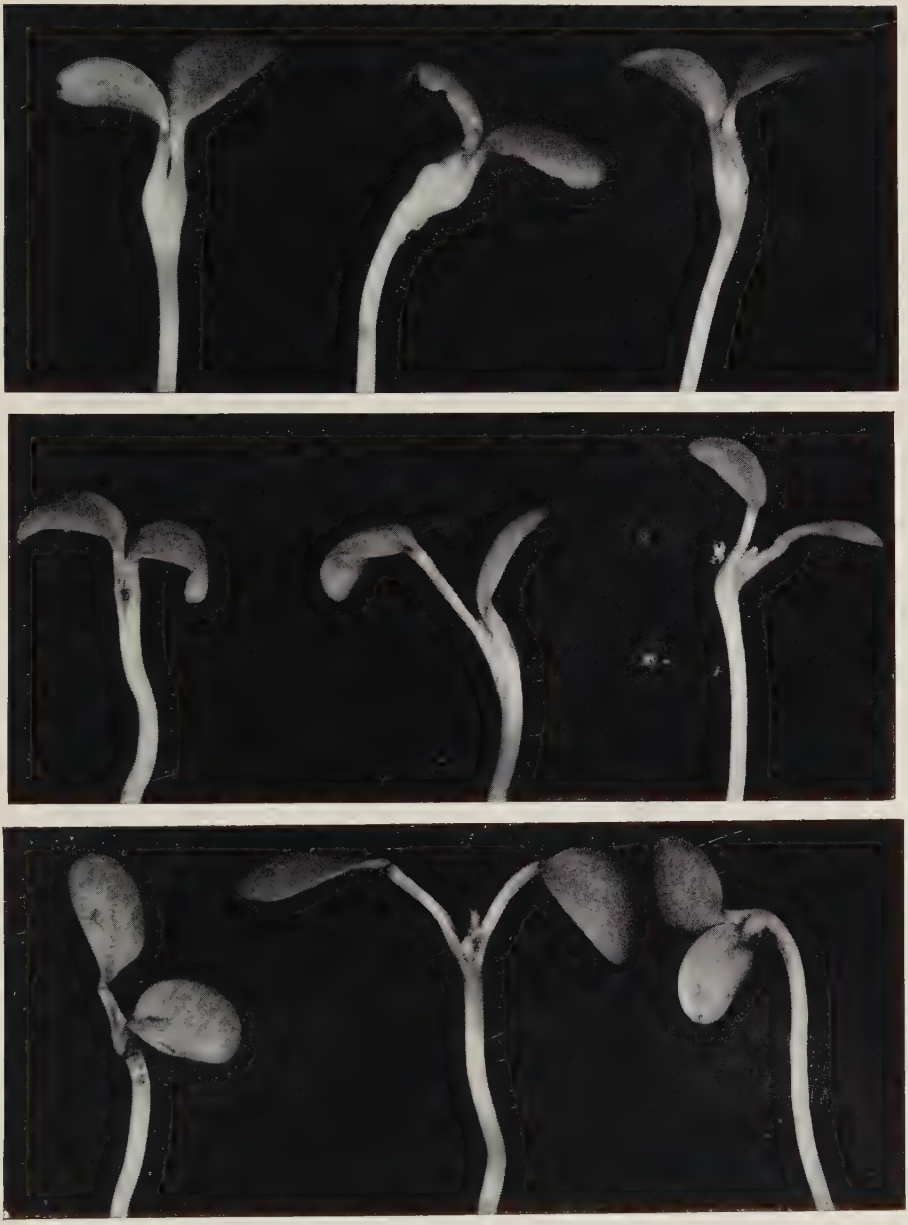
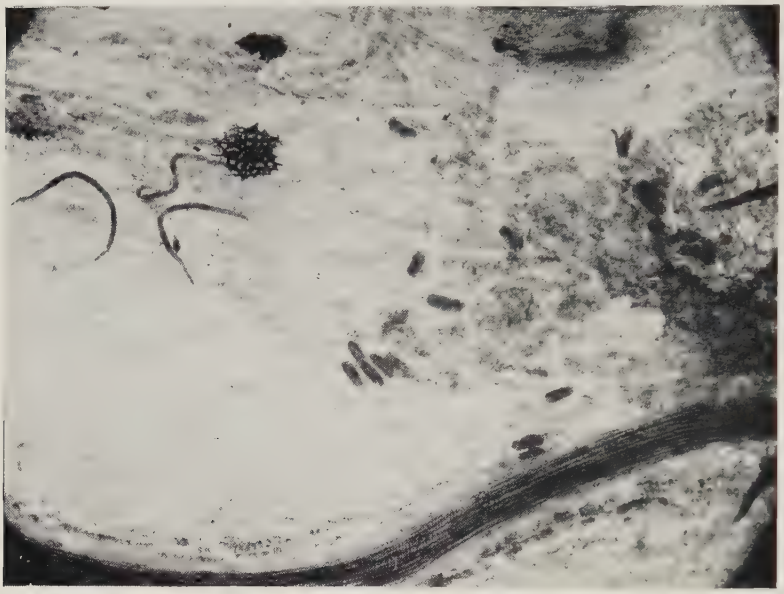


Fig. 1. Seedlings of the variety Merkur, ten days old, inoculated with red clover eelworms. A. Stunted growth. Abnormal swelling at the growing point, at the base of the cotyledon stalks and at the top of the hypocotyl. No brown stripes. Susceptible. B and C. Stunted growth without abnormal swelling. Brown stripes and distortions. Resistant. (From Euphytica 5 (1956) 298-307).

NEMATOLOGICA, II. — J. DIJKSTRA: Stem Eelworm in red clover.



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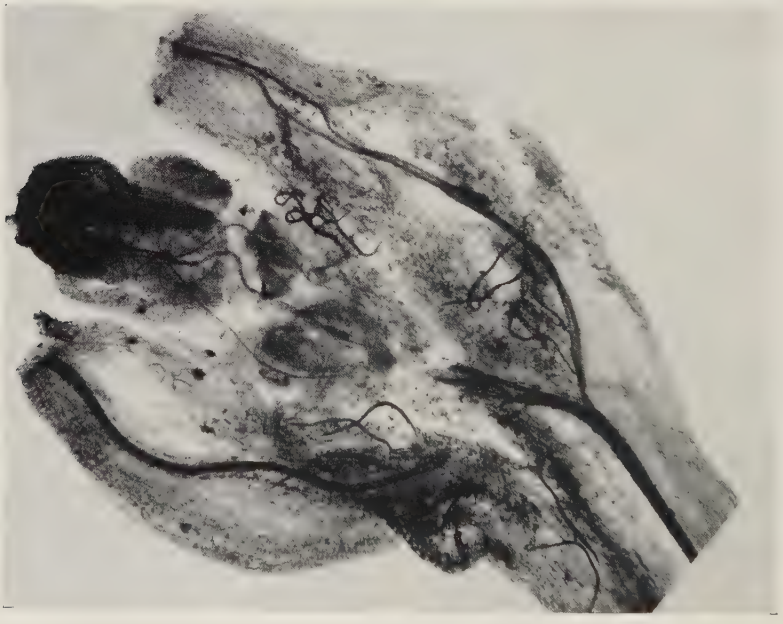

A

NEMATOLOGICA, II. — J. DIJKSTRA: Stem Eelworm in red clover.

C D E

Fig. 2. A. C. and D. Parts near the growing point, cotyledon petiole and part of hypocotyl, 32 days after inoculation. Eelworms stained with cotton blue. A. Stunted growth, greatly swollen containing large eelworms, eggs and small eelworms. Susceptible. B. Enlargement of part of A, showing eggs and small eelworms. Below, parts of large eelworms. C. Healthy looking plant. Normal development of petiole of the first leaf after the cotyledon. D. Stunted growth. Large eelworms within the plants. No abnormal swelling, no eggs or small eelworms present. Resistant. E. Enlargement of part of D.

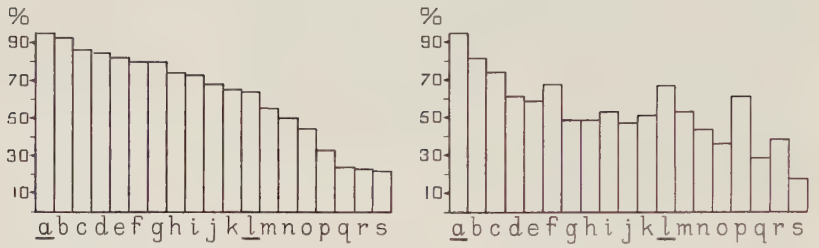

Fig. 3. Above: percentage of galled plants of varieties and families arranged in descending order of these figures. Below: the ratio of greatly swollen plants to the galled ones expressed as a percentage. Roosendaal, Merkur and various families inoculated with the red clover stem eelworm.

a. Roosendaal red clover
b. HaD6
c. HaD1
d. HaD8
e. MaD37-5
f. HaD2
g. HaD3
h. HaD7
i. HaD4
j. MaD6-1
k. HaD5
l. Merkur
m. RoD1-6
n. MaD30-5-5
o. MaD1-2-1
p. MaD45-5-4
q. RoD1-10
r. MaD42-15-5
s. MaD44-19-4

continued to swell and therefore it was easier to separate susceptible and resistant plants after two weeks rather than one.

Fortunately the number of plants showing brown stripes and swellings ((2) and (3) above was too small to upset classification.

4. Brown lesions on swollen plants and general rotting of all plants sometimes occurred about two weeks after inoculation, but could be largely avoided by growing the plants in abundant light at a temperature of 15°-18° C under conditions that were not too moist.

Number of eelworms and eggs in groups of plants with different symptoms. The following groups of plants were chosen sixteen days after inoculation.

1. Greatly swollen plants, the swollen part more than twice as thick as normal.
2. Slightly swollen plants, the swollen part less than twice as thick as normal.
3. Stunted plants with brown stripes and no swellings.
4. Plants of healthy appearance, no swellings, with or without slightly stunted growth.

The plants were obtained from Roosendaal red clover and from families of Red Meuse which had been inoculated when five days old.

Samples of plants from each of the above groups were stained with cotton blue (GOODEY 1949) sixteen and thirty-two days after inoculation. The last group of plants was transferred from the filter paper to soil sixteen days after inoculation. To facilitate counting of nematodes, the cotyledons were removed, and the stained and cleared plants were squeezed between two slides held together by Terry clips. In counting eelworms were divided into large (over ½ mm) and small (under ½ mm) and the number of eggs was also noted. No difficulties were experienced in distinguishing between large and small eelworms. The counts are presented in Table 2 and are arranged in descending order of the number of large eelworms present.

Multiplication of eelworms. With few exceptions, no eggs and small eelworms occurred in the plants sixteen days after inoculation indicating that multiplication of eelworms had not taken place. However, thirty-two days after inoculation multiplication had taken place in swollen plants (Table 2, rows V_5, V_6, V_7 and V_8; Fig. 2 A and B). Comparing rows V_5 and V_6 in the variety Roosendaal red clover it can be seen that the eelworms behaved differently. A Wilcoxon [1]) test (WILCOXON 1945, WABEKE and VAN EEDEN 1955) showed that the

Table II

Number of large eelworms, eggs and small eelworms in groups of inoculated plants with different symptoms. Multiplication of stem eelworms in clover.

Groups of plants			Plant No 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Mean per plant
			16 days after inoculation																				
Roosendaal red clover	Greatly swollen	A_1	47	39	32	32	28	27	23	23	23	22	20	17	16	12	12	4					23.6
		B_1	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0					0.3
		C_1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0					0.0
		V_1	0	0	0	0	0	0	0	0	0	0	0	0.2	0	0	0	0					0.0
	Slightly swollen	A_2	51	36	34	31	30	28	27	26	21	10	10	9	9	9	6	3					21.2
		B_2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					0.0
		C_2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					0.0
		V_2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					0.0
MaD	Stunted plants with brown stripes and no swellings	A_3	20	20	18	15	11	11	11	9	7	7	7	7	7	7	4	4	3	3	3	1	8.8
		B_3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
		C_3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
		V_3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
	Plants of healthy	A_4	8	8	7	7	2	2	1	1	0	0	0	0	0	0	0	0					2.3
		B_4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					0.0
		C_4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					0.0

Roosendaal red clover	swollen	C_5	6	7	5	0	21	4	25	1	2	2										7.3
		V_5	4.4	2.7	3.0	3.9	3.3	4.5	12.2	4.5	7.5	7.5										4.4
	Slightly swollen	A_6	22	19	15	13	13	13	13	8	6	4	3									11.8
		B_6	33	13	47	6	12	22	28	12	19	10	11									19.4
		C_6	2	0	2	2	0	0	5	0	7	6	0									2.2
		V_6	1.6	0.7	3.3	0.6	0.9	1.7	2.5	1.5	4.3	4.0	3.7									1.8
MaD	Greatly swollen	A_7	28	11	11	8																14.5
		B_7	32	24	39	50																36.3
		C_7	6	11	0	5																5.5
		V_7	1.4	3.2	3.5	6.9																2.9
	Slightly swollen	A_8	23	16	16	15	14	13	9	8	6	5	4									11.8
		B_8	34	44	24	28	30	23	45	11	10	12	22									25.7
		C_8	0	14	2	0	6	10	0	2	0	0	17									4.6
		V_8	1.5	3.6	1.6	1.9	2.6	2.5	5.0	1.6	1.7	2.5	9.9									2.6
MaD	Stunted plants with brown stripes and no swellings	A_9	16	14	13	12	12	12	11	9	9	9	8	8	8	5	4	4	4	3	2	8.6
		B_9	0	0	11	0	7	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1.0
		C_9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
		V_9	0	0	0.8	0	0.6	0	0	0	0.1	0	0	0	0	0	0	0	0	0	0	0.0
	Plants of healthy appearance	A_{10}	7	6	3	2	1	1	0	0	0	0	0									1.8
		B_{10}	9	0	12	0	0	0	0	0	0	0	0									1.9
		C_{10}	0	0	0	0	0	0	0	0	0	0	0									1.1
		V_{10}	1.3	0	4	0	0	0														0.1

Key: A large eelworms per plant
B eggs per plant
C small eelworms per plant

$$V = \frac{B + C}{A}$$

MaD Families derived from Red Meuse clover

rate of multiplication of eelworms in greatly swollen plants was significantly higher than in the slightly swollen (P = 0.02). The same holds for the greatly swollen plants of Roosendaal red clover compared with the slightly swollen plants from resistant families of Red Meuse clover (rows V_5 and V_8). In non-swollen plants with stunted growth and brown stripes (Table 2 row V_9. Fig. 2 D and E), no multiplication had occurred.

Penetration of eelworms. There was a clear difference between the number of eelworms penetrating swollen plants and stunted ones with brown stripes sixteen days after inoculation. The mean figures for the first groups were 23.6 and 21.2 (Table 2, row A_1 and A_2) and for the second 8.8 (row A_3). Thirty-two days after inoculation the number of large eelworms in swollen plants had became lower. There is a systematic difference, which the Wilcoxon test showed to be significant for greatly swollen plants of Roosendaal red clover at sixteen and thirty-two days (P = 0.01) or for slightly swollen plants between the same times (P = 0.025), (compare rows A_1, with A_5 and A_2 with A_6). In contrast there was no difference between the stunted plants with brown stripes at sixteen and thirty-two days (row A_3 and A_9).

Among the healthy-looking plants the rate of penetration was low. About half contained no eelworms and several others very few (Table II, A_4 and A_{10}. Fig. 2 C).

Discussion. Multiplication of the eelworms occurs in the swollen plants only whereas in non-swollen plants with stunted growth and brown stipes no multiplication takes place. Clearly the swollen plants are susceptible and the others resistant. This is in accordance with SEINHORST's observations. (1956). The higher rate of multiplication in the greatly swollen plants of Roosendaal red clover compared with that in the slightly swollen plants of the same variety and of the families

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1) "Wilcoxon's two samples test" is based on the computation of a quantity W as follows. Arrange the numbers of the samples in ascending order and underline the numbers from one sample. Thus for rows V_5 and V_6, with V_6 underlined, we obtain:

0.6, 0.7, 0.9, 1.5, 1.6, 1.7, 2.5, 2.7, 3.0, 3.3, 3.3, 3.7, 3.9, 4.0, 4.3, 4.4, 4.5, 7.5, 7.5, 12.2.

Compute W as twice the number of times figures not underlined are placed before underlined. Working from right to left:

$$W = [(1 \times 2) + (3 \times 4)] \times 2 = 28$$

Under the null hypothesis, the quantity W has a well known distribution that has been tabulated. W as calculated above is critically small and therefore the null hypothesis is rejected.

Wilcoxon's test is to be preferred to Student's t-test if it is not sure that the samples are drawn from a normal distribution.

of Red Meuse clover, is a further indication that the degree of swelling is correlated with the degree of susceptibility. Swelling is independent of the number of large eelworms in the plants because these numbers are about the same for greatly and slightly swollen plants. Some large eelworms appear to have left the susceptible plants between sixteen and thirty-two days after inoculation, while the number of large worms in the resistant plants remained the same.

Stunted plants with brown stripes resist both multiplication and, to some extent, penetration of eelworms. Many of the healthy-looking plants contained no eelworms or very few. Either these plants have escaped or have resisted invasion. The proportion of healthy-looking plants of Merkur in table 1 (5 %) does not correlate with the resistance of Merkur (obtained in Sweden by natural selection) in our field trials. Correlation with proportion of non-swollen plants with stunted growth (31 %) is however quite good. Therefore resistance against invasion is considered of little importance; uninvaded plants had probably escaped infection. Reinoculation of healthy plants is necessary however to test this point.

Bingefors (1957) used a different method of inoculation. He placed a drop of eelworm suspension between the cotyledons of the red clover seedlings (outside the filter paper) and found that two or three days after inoculation the resistant variety Merkur had about 50 % of plants free of eelworms whereas all plants of the susceptible variety Ultuna were invaded. In this case resistance of a high proportion of Merkur plants to invasion is very clear.

In breeding eelworm-resistant red clover the susceptibility of the families is assessed from the percentage of swollen seedlings after inoculation. This procedure is also practised by Frandsen (1951) and by Bingefors (1952 and 1957). Galled plants can be counted with greater speed than ungalled plants with stunted growth and brown stripes. However the latter type of plants is preferable to healthy-looking plants for further breeding as it gives greater certainty that resistant plants have been selected.

ACKNOWLEDGEMENTS

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ZUSAMMENFASSUNG

Von Rotkleepflanzen, die auf Filtrierpapier mit Rotkleestengelälchen (*Ditylenchus dipsaci*) infiziert worden waren, konnten folgende Gruppen aufgestellt werden: a) stark geschwollene Pflanzen, b) schwach geschwollene Pflanzen, c) nicht geschwollene Pflanzen mit Wachstumshemmung und braunen Strichen und d) gesund aussehende Pflanzen. In einzelnen Fällen machte die Einteilung in Gruppen Schwierigkeiten.

Die Vermehrung der Älchen in diesen Gruppen wurde 16 und 32 Tage nach der Infektion bestimmt. 16 Tage nach der Infektion war bei keiner dieser Gruppen eine Vermehrung zu beobachten. 32 Tage nach der Infektion hatten sich die Älchen in den stark geschwollenen Pflanzen (Roosendaalse Rotklee) stark, in den schwach geschwollenen (Roosendaalse Rotklee und von rotem Maasklee herstammende Familien) weniger stark vermehrt, während in den nicht geschwollenen Pflanzen mit Wachstumshemmung und braunen Strichen (von rotem Maasklee herstammende Familien) im allgemeinen keine Vermehrung stattgefunden hatte. In dieser Reihenfolge nimmt die Anfälligkeit also ab. Die Pflanzen der dritten Gruppe können als resistent betrachtet werden. In den gesund aussehenden Pflanzen waren meistens keine oder sehr wenig Älchen anwesend. Wahrscheinlich sind diese Pflanzen nicht oder so wenig infiziert worden, dass sich keine Symptome erkennen liessen.

Das stärkere oder schwächere Anschwellen der Pflanzen war nicht auf stärkere oder geringere Infektion zurückzuführen. Daneben konnte festgestellt werden, dass bei den nicht geschwollenen Pflanzen mit Wachstumshemmung und braunen Strichen weniger Älchen in die Pflanzen eingedrungen waren als bei den geschwollenen Pflanzen und dass ein Teil dieser Älchen die geschwollenen Pflanzen nach einiger Zeit verlassen hatte, was für die nicht geschwollenen nicht zutrifft.

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APHELENCHOIDES CITRI N. SP., EIN NEUER WURZELPARASIT DER ZITRONE [1])

VON

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Die im folgenden besprochene neue Nematoden-Art entstammte von den Wurzeln eines jungen Zitronenbaumes aus einer Versuchsstation in der Umgebung von Budapest. Die Wurzeln der betreffenden Pflanze waren von Exemplaren der Art *Aphelenchoides citri* n. sp. sehr stark befallen, das Bäumchen war offenbar wegen der Schmarotzer in seiner Entwicklung bemerkbar zurückgeblieben. Bezüglich der pathologischen Veränderungen der oberirdischen Pflanzenteile konnte ich leider nichts Näheres erfahren, es war aber festzustellen, dass die Wurzeln ungewöhnliche, kleine Verdickungen trugen. Diese Verdickungen waren an den von den Nematoden am stärksten befallenen Teilen der Wurzeln zu beobachten. Es muss jedoch bemerkt werden, dass die Exemplare der erwähnten *Aphelenchoides*-Art nicht nur im Gewebe und an der Aussenfläche der Wurzeln gefunden wurden, sondern eine grosse Menge davon sich auch in der Rhizosphäre des Zitronenbaumes aufhielt. — Die Beschreibung der neuen Nematoden-Art ist wie folgt (Abb. 1-2).

Masse: (n = 5 ♀) L = 0,427-0,476 mm; a = 30,4-34,0; b = 7,5-8,2; c = 4,0-4,2; V = 63,1-64,6 %.

Kopf abgerundet, kaum abgesetzt, Lippen schwach entwickelt. Kutikula dünn, fein aber deutlich quergeringelt, Ringelbreite in der Mitte des Körpers etwa 1 μ. Kopf ungeringelt, mit ziemlich schwachem Versteifungsapparat. Seitenmembran schwer nachweisbar. Mundstachel sehr schlank, seine Länge beträgt 12,9-14,0 μ, ist also 2½-3mal so gross wie die Kopfbreite bzw. ¼ der Entfernung vom Kopfende bis zum Hinterrand des Bulbus. Stachelknöpfe völlig fehlend. Es gibt einen kleinen Führungsring. Vorderteil des Ösophagus schmal, Mittelbulbus etwa zweimal so lang wie breit (12,3-14,0 μ lang, 6,4-7,0 μ

1) Eingegangen: 18. März, 1957.

breit); nach vorne verschmälert er sich ein wenig. Die Chitinverdickung befindet sich hinter der Mitte des Bulbus (konstantes Merkmal). Die Ösophagusdrüsen liegen frei in der Leibeshöhle, sind ziemlich langgestreckt, die hintere ist etwa 1½mal länger (87,7-90,1 μ) als die Entfernung zwischen dem Vorderende des Körpers und dem Hinterrand

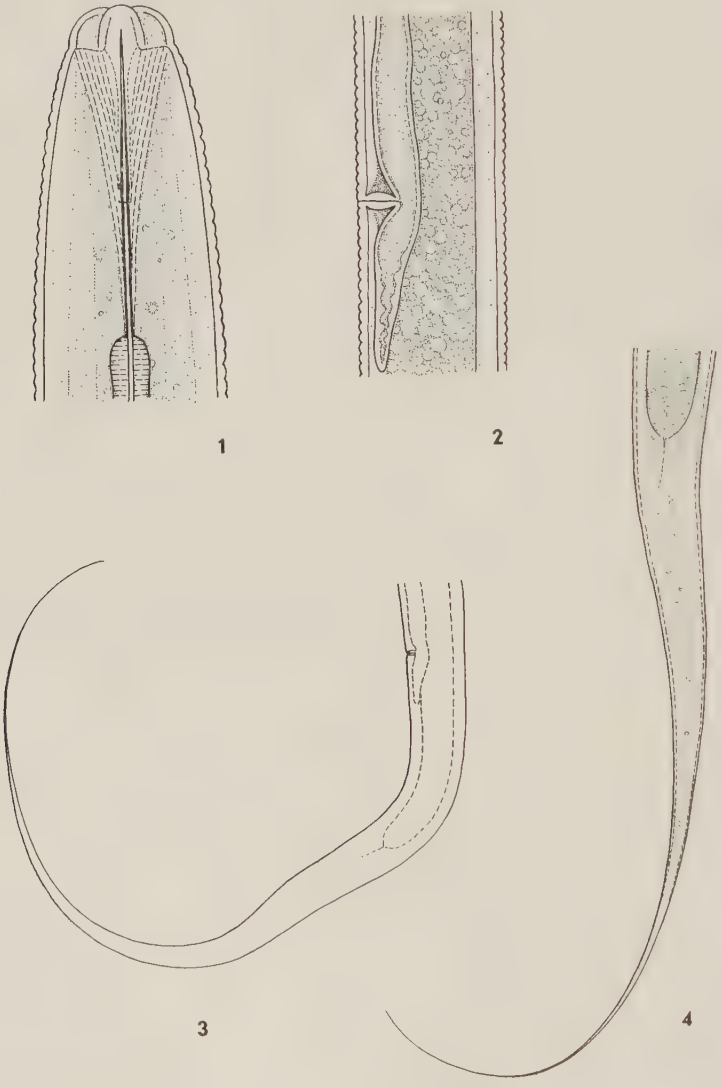

Abb. 1. *Aphelenchoides citri* n. sp.: 1 Vorderende, 2 Vulvagegend, 3 Hinterkörper, 4 Schwanz.

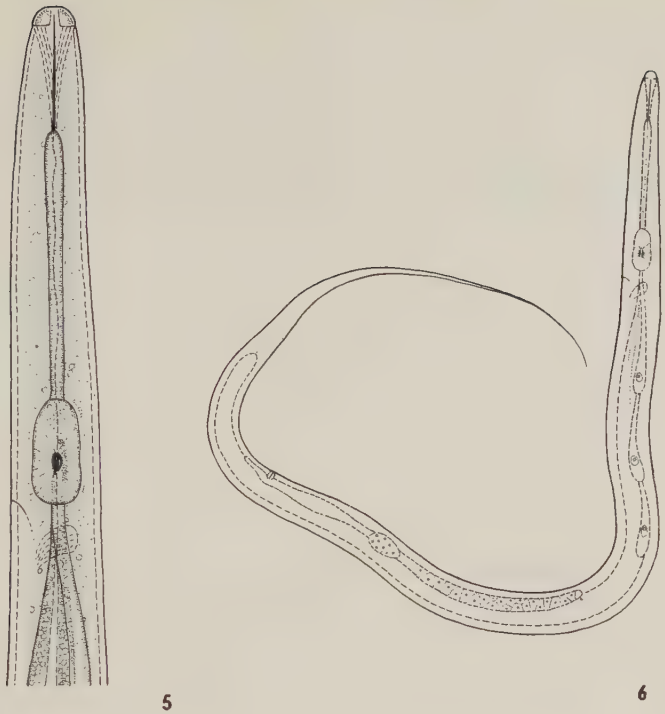

Abb. 2. *Aphelenchoides citri* n. sp.: 5 Vorderkörper, 6 Totalansicht eines ♀.

des Mittelbulbus. Exkretionsporus im allgemeinen knapp hinter dem Bulbus. Darm stark granuliert, Enddarm dünn, schwer sichtbar, länger als die anale Körperbreite.

Die Vulva befindet sich ein wenig vor dem hinteren Körperdrittel, ihre Lippen sind klein, nicht chitinisiert. Vagina kurz, erreicht höchstens $^1/_3$ der entsprechenden Körperbreite. Ovarium gestreckt, verhältnismässig kurz, endigt nach vorne stets hinter der Ösophagusdrüse. Uterus schmal, ein kurzer postvulvarer Uterusast ist vorhanden (er ist nie länger als der betreffende Körperdurchmesser).

Schwanz sehr lang, etwa $^1/_4$ der Körperlänge bzw. 12-13mal grösser als die Analbreite, ausserordentlich fein ausgezogen, so dass das Ende des Schwanzfadens auch durch Immersion kaum sichtbar ist. — Männchen unbekannt.

Diagnose: *Aphelenchoides* mit kaum abgesetztem Kopf, fein geringelter Kutikula, langem und schmalem, nicht geknöpftem Mundstachel, länglichem Mittelbulbus, langgestreckten Ösophagusdrüsen, undeutlichem Rektum, hinterständiger Vulva, verhältnismässig kurzer

Gonade, kleinem hinterem Uterusast und langem, sehr fein verjüngtem Schwanz.

Typus: 1 ♀ mit folgenden Massen: L = 0,442 mm; a = 30,4; b = 7,7; c = 4,0; V = 63,5 %. Paratypi: 17 ♀. Typus und Paratypi befinden sich in der Sammlung des Verfassers.

Typischer Fundort: Zitronenwurzeln in Budapest.

Die neue Art steht den Arten *Aphelenchoides tenuicaudatus* (DE MAN, 1895) T. GOODEY, 1933 und *A. demani* (T. GOODEY, 1928) T. GOODEY, 1933 am nächsten. Vom *tenuicaudatus* unterscheidet sie sich durch ihre kleinere Gestalt, den kaum abgesetzten Kopf, den kleineren Mundstachel und den viel längeren, haarfein ausgezogenen Schwanz, vom *demani* weicht sie durch den schwach abgesetzten Kopf, den mehr gestreckten Ösophagusbulbus, das Vorhandensein des hinteren Uterusastes und den noch feiner verjüngten Schwanz ab.

SUMMARY

Aphelenchoides citri n. sp. was found on the roots of a young lemon tree. Diagnosis: Head slightly offset; cuticle finely striated; lateral fields inconspicuous; spear long without basal knobs; bulb elongated, about twice as long as wide; esophageal glands free, slender; rectum longer than the anal body diameter, fairly indistinct; vulva at about two-thirds of body-length; ovary relatively short, a posterior uterine sac present; tail very long and filiform; male unknown.

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Nematologica II (1957) : 242-249

PRELIMINARY STUDIES ON THE EFFECT OF ETHYLENE DIBROMIDE FUMIGATION ON THE HATCHABILITY OF *HETERODERA ROSTOCHIENSIS* (WOLL.) [1])

BY

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Although the literature on soil fumigation is extensive, very little work has been undertaken on "*in vitro*" effects of chlorinated hydrocarbons on cysts of the potato root eelworm. In most cases, workers have estimated the kill empirically by exposing cysts to the action of potato root diffusate and comparing the hatch from control and from fumigated cysts (PETERS & FENWICK 1949, PETERS 1955, STONE 1956). The form and relationships of the hatching curve have been investigated and methods of conducting hatching tests for assay purposes have been described (FENWICK 1950, FENWICK & WIDDOWSON 1957) but no information is as yet available on the effect of fumigation on the form of the hatching curve; nor is there any valid reason for assuming that "hatchability" under laboratory conditions bears any relation to viability. This paper records preliminary observations on cysts exposed to the vapour of ethylene dibromide and hatched in potato root diffusate; the main object of the work was to determine the effect of such fumigation on the form of the hatching curve and to determine whether or not the hatching technique described by FENWICK & WIDDOWSON was applicable to fumigated cysts.

Material and Methods

The cysts were drawn from a common stock recovered from soil in the ordinary way (FENWICK & WIDDOWSON 1957). For fumigation, they were enclosed in 1½-in. lengths of 8 mm. glass tubing, the ends of which were plugged with non absorbent cotton wool; the

1) Received for publication: March 22, 1957.

tubes with their contained cysts were suspended in air tight jars of about 1 litre capacity. Fumigation was carried out at a relative humidity of 100 %, achieved by a half inch layer of water in the bottom of each jar. The cysts were suspended in these jars for 48 hrs. at the experimental temperatures before fumigation to equilibrate them to the conditions involved. Fumigation was done by introducing the fumigant into the jar in an open solid watchglass which was stood in the water; when the fumigation time had elapsed the jar was opened, and the cysts transferred for 48 hrs. to an open watchglass and exposed to the air to dispel any fumigant left on them. They were then stored for one or two months, replicate batches of 100 cysts were picked out, presoaked and immersed in potato root diffusate. Periodic counts were made by dilution on larvae emerging, and sigmoid curves were fitted to the cumulative data thus obtained. A further test was usually carried out two or three months later.

Procedure

In a preliminary experiment cysts were exposed to a saturated atmosphere of ethylene dibromide at 100 % humidity for one hour at room temperature. A hatching test was carried out two months after treatment and was continued until hatching had ceased. Replication was five-fold; the diffusate used had an L.A. value of 2.9; total hatch from control cysts represented 90 % of the total contents. Hatching curves for treated and untreated cysts are shown in Fig. 1, the sigmoids being derived by back plotting from probit lines drawn by eye. The total emergence at the end of the experiment was 4,515 and 2,835 larvae from control and fumigated cysts and represented a loss of 37 % in hatchability. The points of inflection of the curves occur at 7¼ and 28 days respectively. Estimates based on hatch at 1, 2, 3, 4 and 5 weeks would give losses of 81, 68, 58 and 52 % respectively and would have exaggerated the effect of fumigation.

A further test was carried out at 15, 20, 24 and 30° C for 1, 2 and 4 hours. Emergence in the control series approximated to 85-90 % of the total cyst contents; the final hatch from the treated cysts expressed as a percentage of that from the controls are given in Table I. Sigmoids were plotted for this data and the points of inflection interpolated and it was found that they were later for treated cysts than for controls. To investigate relationships between the point of inflection and decrease in hatchability the former were plotted against the latter and the graph obtained is given in Fig. 2 and it is seen that

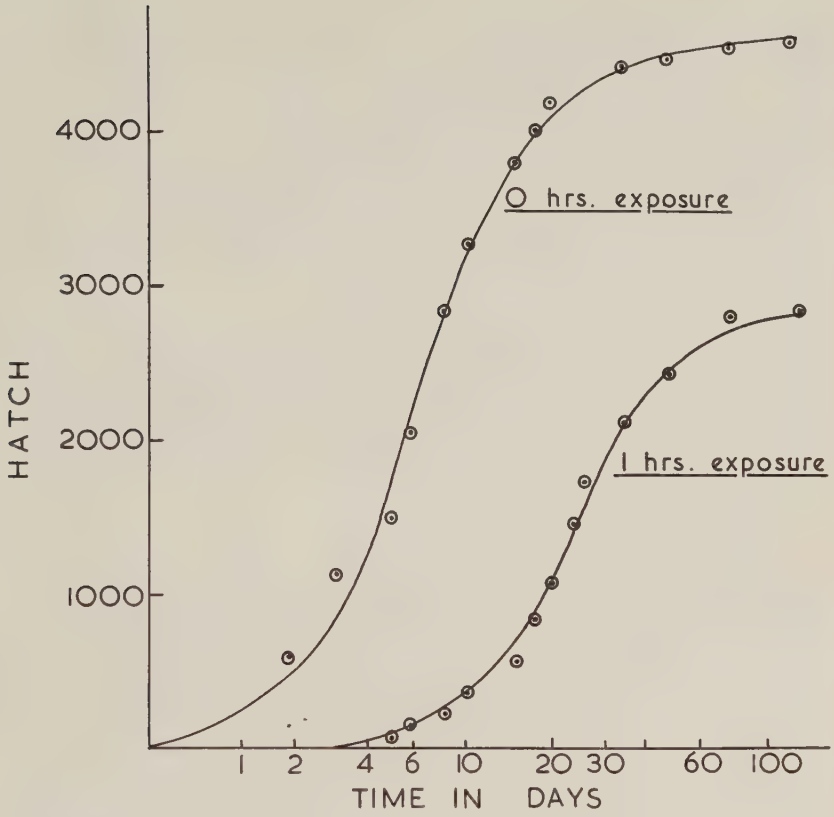

Fig. 1. The effect of ethylene dibromide fumigation on the hatching curves of cysts of *Heterodera rostochiensis.*

TABLE I

Total hatch from cysts exposed to ethylene dibromide for different times at different temperatures. Hatch expressed as a percentage of that for untreated cysts.

Treatment time	Treatment temperature			
	15°C	20°C	24°C	30°C
1 hr.	96	66	51	2.4
2 hr.	74	16	1.5	<1
4 hr.	67	<1	<1	<1

decrease in hatchability tends to be associated with a high time value for the point of inflection. Estimates based on hatching over a fixed

time would therefore have been misleading: for fumigation at 15° C the final estimate indicated losses of 4, 26 and 33 % in hatchability for 1, 2 and 4 hrs. treatment but had estimates been made earlier the results would have indicated losses of 19, 41, and 85 % for three week data and 7, 31 and 68 % for four week data.

Another test was carried out after a further three months storage but reduction in hatch was far greater than in the first test. The data is set out in Table 2. Study of the form of the curves obtained was

TABLE II

Total hatch from cysts treated as in Table I but stored for 4 months before testing. Hatch expressed as a percentage of controls.

	Treatment Temperature			
Treatment time	15°C	20°C	24°C	30°C
1 hr.	32	2.4	7.1	1.7
2 hr.	3.7	<1	<1	<1
4 hr.	1.5	<1	<1	<1

difficult due to the very low level of emergence except in the case of 1 hr. treatment at 15° C: in this case the points of inflection for control and treated cysts were reached in five and 15 days respectively.

In a third experiment cysts were treated as in the previous experiment but for ½, ¾, 1, 2, 4 and 8 hrs. at 15° C and 20° C. The first hatching test occurred 1 month after treatment. Larval emergence from controls was 86 % of the total contents but larval emergence from the cyst treated for short periods appeared to be higher than from the controls. This was found to be due to the control cysts having fewer contents than the treated. This was corrected and the data in Figs. 3 and 4 for 15 and 20° C carry this correction. Although up to 1 hr. treatment has had very little effect on final hatch the form of the hatching curve has been considerably altered and the time for attaining the point of inflection was increased. The effect of fumigation on this is set out in Table 3. The vertical lines drawn correspond-

TABLE III

Effect of fumigation on the point of inflection of hatching curves.

		time in hours					
	Control	½	¾	1	2	4	8
15°C	6.4	10.7	9.9	10.7	12.8	14.1	17.2
20°C	9.6	15.9	16.3	16.4	21.4	25.9	26.9

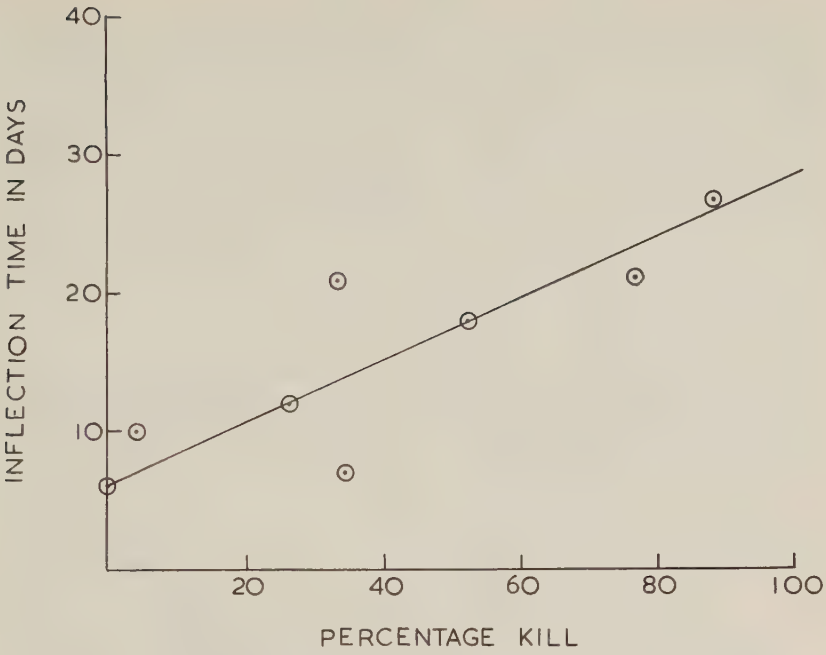

Fig. 2. The relationship between the point of inflection and decrease in hatchability.

ing to 1, 2, 3 and 4 weeks on the graphs of Figs. 3 and 4 show how misleading estimates would have been obtained if the hatching tests had been carried on for these respective times only.

A further test on these cysts was made two months later and the total larval emergence from them is presented in Table 4 and the effect of fumigation on the point of inflection in Table 5. Again it is

TABLE IV

Total hatch from cysts exposed to ethylene dibromide for different times at 15° C and 20° C. and stored 3 months before testing. Hatch expressed as a percentage of control

	time in hours					
	½	¾	1	2	4	8
15°C	96	99	91	77	31	18
20°C	26	12	14	17	14	10

TABLE V

Effect of fumigation on the point of inflection of hatching curves for cysts from Table IV.

	time in hours					
	½	¾	1	2	4	8
15°C	7.3	9.9	11.3	13.3	15.2	14.4
20°C	8.2	10.2	12.5	11.7	10.0	11.3

seen that there has been a change in the latter although it is not so marked as in the previous cases, nevertheless, considerably exaggerated estimates of the effect of ethylene dibromide would have been obtained had this test been cut short.

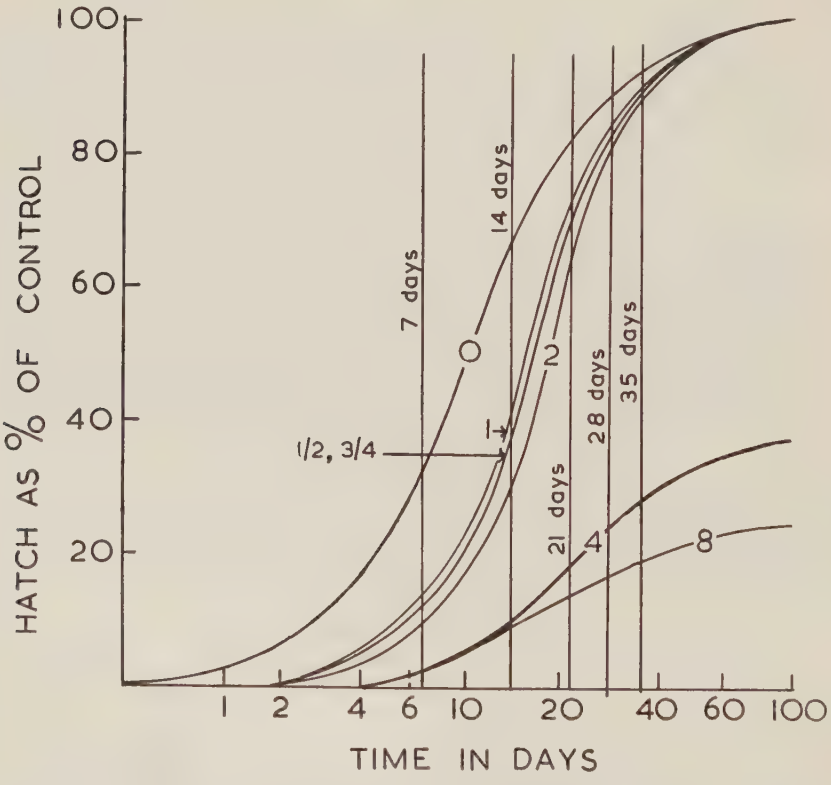

Fig. 3. Hatching curves for cysts of *Heterodera rostochiensis* exposed for varying times to ethylene dibromide at 15° C.

Discussion and Conclusions

The data suggest that fumigation of cysts with ethylene dibromide, in addition to reducing their hatchability also affects the form of the hatching curve by increasing the time necessary to attain the point of inflection. It appears from Fig. 2 that there is some degree of correlation between the percentage reduction in hatchability and the time corresponding to the inflection point, although Figs. 3 and 4

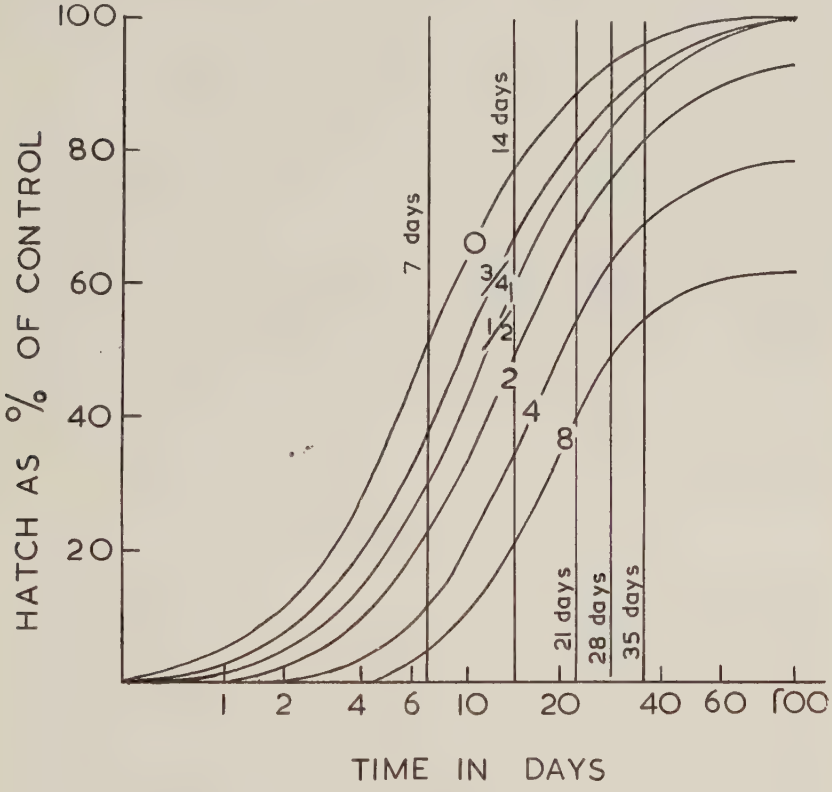

Fig. 4. Hatching curves for cysts of *Heterodera rostochiensis* exposed for varying times to ethylene dibromide at 20° C.

suggest that the form of the hatching curve can be altered even in the absence of any decrease in total hatch. This shift in the point of inflection and the consequent change in the form of the hatching curves precludes the possibility of comparing hatches from treated and from control cysts unless the test is carried on to completion or the form of the curve investigated. A comparison of Tables III

and V suggests that if cysts are allowed to stand for some time after treatment the alteration in the point of inflection is less than if they are tested relatively soon after treatment. Even so, after three months delay it is still dangerous to draw conclusions from the results of tests continued for a limited time.

There appear to the author to be two ways of overcoming this difficulty. If a shortened test is desirable then the shortened method (FENWICK 1951) could be used. This would involve daily counts when the point of inflection was passed and extrapolation to determine the final hatch. A better method involving a lesser expenditure of labour would be to carry the hatching test on to completion.

While it is not intended to deal with the action of ethylene dibromide in detail, two features of the results are interesting; firstly, the greater effect of fumigation when carried out at higher temperatures as opposed to lower. In all cases the greatest effect was obtained at 30° C, 1 hr. fumigation at 15° C (Table II) decreased hatch to 35 %, but the same treatment at 30° C reduced it to 1.7 %. If, therefore, hatchability bears any relation to viability, then fumigation with this chemical should be carried out at higher temperatures: secondly the greater decrease in hatchability when cysts are tested some months after treatment (Tables III and V) suggests that the action of the chemical persists long after treatment which is in agreement with the observations of many workers.

ZUSAMMENFASSUNG

Die Wirkung einer Begasung von *Heterodera rostochiensis*-Cysten auf ihre Schlüpffähigkeit wurde untersucht. Ein vorläufiger Versuch wurde bei 20° C und 100 % Feuchtigkeit in einer gesättigten Atmosphäre von Äthylendibromid durchgeführt. Die Schlüpffähigkeit der Larven wurde vermindert und der Umkehrpunkt der Schlüpfkurven trat für begaste Cysten bei 28 Tagen ein im Vergleich zu 7¼ Tagen bei den Kontrollen. Ähnliche Ergebnisse wurden nach Begasung bei 15, 20, 25 und 30° C erhalten. Wenn der Schlüpftest mehrere Monate verzögert wurde, war die Schlüpffähigkeit noch weiter vermindert und die Verzögerung im Erreichen des Umkehrpunktes war noch deutlich. Diese Ergebnisse zeigen, dass Schlüpfversuche, die nur für eine begrenzte Zeit fortgesetzt werden, bei begasten Cysten irreführende Ergebnisse geben können. Es wird empfohlen, dass unter diesen Bedingungen die Prüfung bis zu Ende durchgeführt wird.

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Nematologica II (1957): 250-254

AN INVESTIGATION INTO THE POSSIBILITY OF PARTHENOGENESIS IN THE POTATO-ROOT EELWORM, *HETERODERA ROSTOCHIENSIS* WOLLENWEBER [1])

BY

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Investigation of the sex-ratio of the potato-root eelworm *Heterodera rostochiensis* Wollenweber showed that, under certain conditions, more females than males are found (Ellenby, 1954). In that work, the plants were lifted before the parasites were mature; their fate, particularly that of the supernumery females, was therefore uncertain. Sengbush (1927) first recorded pairing of the sexes in *H. schachtii* and claimed also to have observed spermatozoa in the female after pairing; I have observed pairing in *H. rostochiensis* on numerous occasions and so have other workers (Peters, van der Laan, personal communications) so that it would not be unreasonable to assume that, under normal conditions, fertilization takes place. But parthenogenesis is not unknown among nematodes, and is found in the closely related root knot nematode (*Meloidogyne* sp.), which Tyler (1933) was able to maintain for twelve generations parthenogenetically. Could it occur in the potato root eelworm if, for some reason, a female failed to be fertilized? As recently emphasized by Toxopeus (1956), new biotypes of parasite could be more readily established if parthenogenesis occurs; an answer to the question is of more than academic interest. The possibility was therefore investigated.

Experimental. Forty nine potato plants were raised from cuttings of the variety Arran Banner in sand plus artificials in glass-sided inspection boxes. They were maintained outdoors, in racks, and their glass sides were screened with roofing felt. Half were inoculated three weeks later, and the remainder four weeks after that. Forty five of the plants were inoculated each with a single larva, the remaining four plants receiving three, five, ten, and twelve respectively. The larvae for all plants inoculated had emerged from one single cyst during the

1) Received for publication: March 26, 1957.

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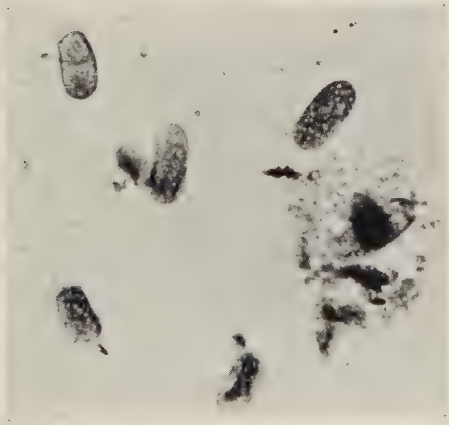

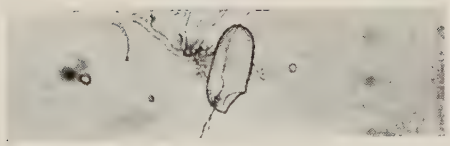

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Fig. 1 & 2. Cyst on plant 29, inoculated with a single larva on June 25. White female first found Aug 27; this had turned ivory colour by Nov 23. In Fig. 1 it is shown on Dec. 31 when it was yellow. Fig. 2 shows the cyst on Jan 11 when it had turned brown. Cyst 0.4 mm. diam. — Fig. 3. A group of eggs from the cyst from plant 18, inoculated with a single larva. Eggs about 90 μ long. — Fig. 4. Empty egg shell found in this condition among eggs from cyst from plant 18. — Fig. 5. Egg from cyst on plant 18 more highly magnified to show apparently normal shell.

previous twenty four hours; they were transferred with a mounted hair to the surface of a selected root, a short distance behind its tip. Inoculation points were marked with small pieces of plastic. The roots were examined from time to time.

On the four plants inoculated respectively with three, five, ten, and twelve larvae, one, one, no, and two females were found: they were normal in every way, all turning brown normally and being filled with embryonated eggs, as confirmed by dissection. On the forty-five plants inoculated with single larvae a total of three females were found: these females, although of normal size, were abnormal in other ways.

All three 'single larva' cysts took much longer to turn brown than cysts on the plants inoculated with more than a single larva. The results are summarised in Table I. Two of the 'single larva' cysts took about 150 days from inoculation to brown cyst. In one case the time was 210 days, this particular cyst still being yellow on 29 December and only turning brown on 11 January (Table I). The time for the control cysts was just over 100 days. Clearly, even though the outdoor temperatures were lower later in the year and might have slowed development, the difference is most striking.

TABLE I

Browning of cysts on 'single larva' and control plants.

	Plant serial number			
	'Single larva'			Control
	18	28	29	11, 42, 48
Date				
May 29	Inoculation			
June 25		Inoculation	Inoculation	Inoculation
Aug. 24	Yellow	White	White	Yellow
Sept. 15	Yellow	White	White	Brown
Oct. 5	Light brown	Ivory	Ivory	
Oct. 29	Dark brown	Ivory	Ivory	
Nov. 23		Brown	Ivory	
Dec. 29			Yellow	
Jan. 11			Brown	

The control cysts were removed from their hosts at the beginning of October, that is, about 100 days from inoculation. They were obviously fully ripe and, as already mentioned, dissection showed them to be filled with embryonated eggs. The 'single larva' cysts, however, were left on their hosts as long as possible; they were obviously lagging behind the controls and it was desirable to provide every

opportunity for them, and any eggs they might contain, to develop.

The cyst on plant No 18 was removed from the roots of the host on 15 November, over 160 days from inoculation. It was kept for some days on moist filter paper in a petri dish, and then opened in water. It contained 41 eggs. The cyst on plant No 28 was removed the day it was found to be brown; it had appeared to be empty, and this was confirmed by dissection. The cyst on plant No 29 was found to contain six eggs when opened in water on 11 January, 210 days after inoculation. The contents of all the eggs was disorganised; but a number appeared to show signs of having undergone some development, their contents showing some resemblance to early cleavage stages (such eggs are shown in Figure 3); but this is by no means certain. The egg shell, however, appears to have been properly formed. Figure 4 shows an empty egg shell, found in that condition in the cyst on plant No 18, and a more highly magnified view of an intact egg is shown in Figure 5. The egg shell also appeared to be normal in colour.

Both lots of eggs from the 'single larva' cysts were retained in water for three weeks after removal from their respective cysts; they showed no signs of developing.

DISCUSSION

The number of cysts obtained from both the control plants and those inoculated with a single larva was very low. The small number of females does not mean that all the other larvae with which the plants were inoculated gave rise to males; the results provide no information on the sex ratio, for routine examination of the roots were carried out with the glass sides of the inspection boxes in place in order to disturb the roots as little as possible and, under these conditions, males would not be revealed. Probably the small number of females was largely due to failure on the part of most of the larvae to penetrate the host tissues. I have found this in unpublished experiments, while Tyler (1933) has commented on the lack of ability to penetrate shown by batches of larvae of the root-knot eelworm. The question has also received more recent attention (Dropkin, 1957).

Can useful conclusion be drawn about parthenogenesis from the present results? Although the number of cysts is so small, there was a very sharp distinction between the cysts formed on the control plants and those found on the 'single larva' plants. All four control cysts developed normally, turning brown in the normal time and filling

with embryonated eggs. On the other hand, the three 'single larva' cysts were all abnormal; they took considerably longer to turn brown, in one case more than twice as long as the controls, and were empty or contained eggs all of which failed to develop properly although given ample time. In spite of the small number of cysts involved, the sharp distinction in the development of the two sets suggests that parthenogenesis does not normally take place. However, as in nematodes the egg shell is largely endogenous (CHITWOOD and CHITWOOD, 1950; ROGER, 1956), some "development" of the egg took place in the absence of fertilization. Aberrant development without fertilization is not uncommon (see review in VANDEL, 1931); but it is doubtful whether it is sufficient in the present case to justify the use of the term "parthénogenèse rudimentaire".

The recent announcement in summary by FASSULIOTIS (1957) is of particular relevance to the present work for he has apparently carried out a parallel study using the tomato as host. Twenty eight plants were inoculated with single larvae, and ten females were obtained; there was evidence of one male. The females were of normal size but empty, and it is concluded that parthenogenesis does not take place; it will be interesting to see the full account of this work [1]).

ZUSAMMENFASSUNG

1. 45 Kartoffelpflanzen wurden einzeln in Beobachtungskästen, die mit zwei Glaswänden versehen waren, gezogen. Sie wurden je mit einer einzigen Larve infiziert. Als Kontrolle dienten Pflanzen, die unter gleichen Bedingungen gezogen wurden, die aber mit je 3, 5, 10 und 12 Larven besetzt waren. Alle Larven stammten aus einer Cyste.

2. Die wenigen Cysten, die auf den Kontrollpflanzen gefunden wurden, waren anscheinend normal; alle enthielten Eier mit Embryonen.

3. Drei Cysten wurden auf den eigentlichen Versuchspflanzen gefunden. Sie waren von normaler Grösse, jedoch in anderer Weise abnorm: eine war leer, eine enthielt sechs, und die dritte 41 Eier mit äusserlich normaler Schale. Der Inhalt schien jedoch desorganisiert zu sein. Einige hatten möglicherweise die ersten Stadien der Entwicklung durchgemacht. Diese 3 Cysten brauchten eine längere Zeit zum Braunwerden als die Kontrollen. Eine Cyste war weiss, als sie im August gefunden worden war; sie wurde erst im folgenden Januar braun.

4. Die Zahl der Cysten an Pflanzen mit nur einer Larve ist sehr klein; alle drei unterschieden sich aber so weitgehend von normalen Zysten, dass man wohl den Schluss ziehen kann, dass Parthenogenese in diesem Tiere nicht stattfindet.

1) T. D. WILLIAMS, School of Agriculture, Cambridge, informs me that he has obtained two cysts, both empty, on potato pieces inoculated with single larvae.

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MARJORIE J. TRIFFITT, D.SC.

obiit 9 March 1957

Born in Glasgow, M. J. Triffitt spent her early years in Islay. Later she was educated at Lancaster Girls' Grammar School and at Bedford College (University of London), whence she graduated with Honours in zoology in 1921. Until 1926 her professional interests were mainly protozoological and her name appears on 12 of the 13 research papers in a short-lived journal called 'Protozoology', issued from the London School of Tropical Medicine in 1925-6. As late as 1932 she writes (with others) on a new protozoan parasite of *Bulinus tropicus,* a vector of schistosomiasis.

In 1926 she joined the group of helminthologists under Leiper at his recently-founded Institute of Agricultural Parasitology at St. Albans. Here for some ten years her attention was focussed mainly on the genus *Heterodera.* In 1927 she writes with J. N. Oldham on *Rhabditis coarctata* and in 1931 she gives the earliest British record of *Meloidogyne* causing damage in field crops: all her other papers are on *Heterodera schachtii,* as it was then called, but her observations cover the 'strains' (now species) found on potatoes, beet and mangolds, peas, oats, carrots, and Sea Marram Grass. She also described microcysts.

Her papers dealt with the anatomy of the parasites and with many aspects of their life history: the number of generations in a season, the rate of cysts turning brown, host ranges, the necessity of oxygen for larval hatching. On the pathogenicity of *H. rostochiensis* she was always alert to notice parasitic fungi which might prove pathogenic, and she observed such host reactions as the growth of giant cells in the central stele of the root, with the consequent formation of new lateral roots.

Along with the chemist, R. H. Hurst, she investigated the thermal death point in connection with the hot-water treatment of infested potato tubers, and carried out numerous field experiments on the treatment of infested soils with such substances as calcium cyanamide, ferric oxide, or potassium ethyl xanthate.

Perhaps her best work lay in her investigations of the root diffusates which stimulate or inhibit the hatching of *H. rostochiensis.* She established their production only during the growing season, their rapid destruction under non-sterile conditions, and their relative heat resistance. Winter dormancy, mustard inhibition, and many other aspects were covered.

Nematologists can savour, in the Journal of Helminthology, the high quality of all her work; she was a shrewd, patient, and careful observer with a highly critical mind. But those who were privileged to know her personally (as the writer was from 1926) will rather think of her as a staunch friend who could always be relied upon for help and also, if asked, for a completely honest opinion. What the stranger might miss, because she hid it through shyness, was her very great kindness and warmth of heart.

She was married in 1931 and is survived by her husband, His Honour Judge James MacMillan (a name which explains the letters M. MacM. on some publications of the Imperial Bureau of Agricultural Parasitology), and also by a married daughter.

B. G. P.

NEMATOLOGICAL NEWS

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Change of address:

Winslow, R. D. *now*: Nematology Dept., Rothamsted Exp. Station, Harpenden Herts, England.

GENERALIA

F. G. W. Jones, Rothamsted Experimental Station, Harpenden asks to give notice of the following summary of investigations on *Heterodera rostochiensis*: R. Riley and Chapman working with Dr. H. H. Howard of the Plant Breeding Institute, Cambridge, have counted the chromosomes of *Heterodera rostochiensis*. The number is $2n = 18$; both meiotic and mitotic divisions were seen. The chromosome number and appearance is the same in normal and resistance-breaking populations, although few specimens of the latter were prepared.

A. H. Meyl

Nematologica II (1957): 257-272. — Leiden, E. J. Brill

SOIL POPULATIONS OF BEET EELWORM (*HETERODERA SCHACHTII* SCHM.) IN RELATION TO CROPPING. III FURTHER EXPERIMENTS WITH MICROPLOTS AND WITH POTS [1])

BY

F. G. W. JONES

Rothamsted Experimental Station Harpenden

Early work on soil populations of beet eelworm (*Heterodera schachtii* Schm.) was done in the Chatteris area, Isle of Ely, using two-acre field plots sampled on a fixed grid system (JONES 1945). Later, to exercise more control over variables, a microplot technique was developed (JONES 1955, 1956). Below, the results of a rotational experiment in microplots containing Chatteris soil are given along with brief criticisms of the layout which may be of value to those planning similar experiments. Further results are also given of the effects of beet and other host plants on the beet eelworm population of three sets of microplots containing soil from Oxlode, Spalding and Cantley respectively and of a separate experiment employing large pots. Details of the construction and management of microplots have already been described (JONES 1956). Information concerning the Chatteris, Oxlode, Spalding and Cantley soils are summarized in Table I and the mechanical analyses of the soils are shown in Table II.

The four soils are fairly representative of soils on which sugar beet is grown extensively in Eastern England. Thus, the Chatteris soil is a shallow peat overlying clay from the edge of the black fen basin, the Oxlode soil is a deeper peat overlying clay also from the black fen basin, and the Spalding soil is a very fine sand from the fen silt area, while the Cantley soil is a coarser sand from Norfolk.

The Oxlode soil contained a very heavy natural infestation of beet eelworm and the uniformity of the population obtained by mixing was partly destroyed by the various crops (fallow, host and non-host) grown in 1954 (JONES 1956, Table III). The Spalding and Cantley soils were obtained from fields in which natural infestations of beet

1) Received for publication: January 31, 1957.

TABLE I

Information concerning microplot soils

Locality from which taken	Chatteris, Isle of Ely.	Oxlode, Isle of Ely.	Spalding, Lincs.	Cantley, Norfolk.
Mixed and set up	1951	1954	1954	1954
Crop on field from which taken	Potatoes	Sugar Beet	Potatoes	Wheat
Known contaminants	Cereal root eelworm [2]	Potato root eelworm [1]	Potato root eelworm [1]	Cereal root eelworm [2] Cabbage root eelworm [3]
Original beet eelworm population	56 eggs/g.	480 eggs/g.	Negligible	Negligible
Inoculated with Oxlode soil to give	—	—	1 egg/g.	1 egg/g.
Crops grown	Various, in rotational experiment	Various hosts and non-host crops	Sugar beet, 20 tons/acre	Sugar beet, 20 tons/acre
Mean beet eelworm populations at start of experiments described herein	56 eggs/g.	242 eggs/g.	21 eggs/g.	Approx. 1 egg/g.

1) *Heterodera rostochiensis* Woll. 2) *H. major* O. Schmidt.
3) *H. cruciferae* Franklin.

eelworm were known to occur but, on testing, the level was found to be negligible, no hatch being obtained from cysts extracted from the Cantley soil when subjected to mangold root diffusate and only a small hatch from the cysts from the Spalding soil. Potato root eelworm as a contaminant in the Oxlode and Spalding soils presented no difficulties in cyst and egg counts. There was some difficulty with cysts of cereal root eelworm in the Chatteris soil and with cereal root eelworm and cabbage root eelworm in the Cantley soil. The population levels at the start of the experiments described below are shown at the foot of Table I. It will be noted that there had been a rise after beet in the Spalding soil from 1 to 21 eggs/g., no detectable change in the Cantley soil and a general fall in the Oxlode soil from 480 to 242 eggs/g.

In the laboratory it is usual to attempt to hold constant all factors known to affect an experiment except the factor of which the effects are to be studied. The same degree of control is not usually possible in out door experiments with growing plants. However, in the experiments on the effects of hosts in different soils outlined below attempts to

TABLE II

Mechanical analyses of microplot soils

Source	Agricultural type	Textural class	pH	% by weight — particle size					
				$\%CaCO_3$	2.0-0.2 mm Coarse sand *Grobsand*	0.2-0.02 mm Fine sand *Feinsand*	0.02-0.002 mm Silt *Schluff*	below 0.002 mm Clay *Ton*	Organic matter % *OrganischeMasse*
Chatteris Isle of Ely	Fen skirt (*Grenze des schwarzen Marschbodens*)	Peaty Clay (*Humoser Ton*)	8.1	6.7	1.4	7.7	15.8	57.1	8.8
Oxlode Isle of Ely	Black Fen (*Schwarzer Marschboden*)	Peaty clay (*Humoser Ton*)	7.4	1.0	3.1	23.8	11.0	37.0	17.1
Spalding Lincs.	Fen silt (*Marschschluffboden*)	Fine sand (*Sandig-toniger Lehm*)	7.9	1.3	0.8	74.4	5.1	18.2	1.4
Cantley Norfolk	Sandy Loam (*Sandiger Lehm*)	Fine sand (*Grobsandiger Lehm*)	8.1	1.3	23.6	53.7	3.5	13.4	0.9

control some variables were made. After each one had been homogenised by mixing, all the soils were placed in adjacent blocks of microplots situated within a small area subject to the same macroclimatic conditions. The plots of Spalding and Cantley soils were then uniformly inoculated with Oxlode soil to give 1 egg/g. (JONES 1956). As a result, the soil populations at the beginning of the experiments were more uniform than they would be under field conditions and, except for the plots containing Chatteris soil used in the rotational experiment, the effective eelworm population was derived from one source, the Oxlode soil. In addition, there was an attempt to control plant numbers and to regulate, within limits, the vegetative period of some of the plants. Under these circumstances the chief differences remaining between plots would be the plants grown and the physical, chemical and biological properties of the various soils, including nematode enemies.

Rotational Experiment

The object of this experiment was to follow changes in the beet eelworm population of Chatteris soil when sugar beet (*Beta vulgaris* L.) was grown at various intervals, the rotational treatments being:

(1) Continuous beet
(2) Beet every other year
(3) Beet every three years
(4) Beet every four years
(5) Beet every five years
(6) Beet every six years
(7) Beet followed by grass for five years
(8) Beet followed by fallow for five years.

For treatment (6), the following rotation was employed: carrots (*Daucus carota* L.), barley (*Hordeum sativum* Pers.), chicory (*Cichorium intybus* L.), wheat (*Triticum vulgare* Vill.), potatoes (*Solanum tuberosum* L.), beet, while treatments (5) to (3) were derived from this rotation by successive elimination of the first crop. To avoid building up populations of cereal or potato root eelworm, the sequence of crops used for treatment (2) was beet, potatoes, beet, wheat. All treatments began with the cultivation of sugar beet. There were two replicates of each treatment (16 plots) per block, randomized independently, and three blocks were started in the years, 1952, 1953, and 1954. While awaiting treatment, block No. 2 was held fallow for one year and block No. 3 was held fallow for two years.

After the experiment had run for 4 years, certain inherent weaknesses in design became apparent: (1) The soil contained a low population of cereal root eelworm which tended to increase whenever

cereals were grown which complicated the assay of the egg populations of beet eelworm. (2) As the rotational treatments proceeded, the replication for individual crops was reduced with the result that the population estimates carried progressively less weight (for the actual number of plots for each kind of treatment, see Fig. 1, block No. 1). (3) Under non-host crops the population levels fell and became more difficult to measure accurately. The first difficulty, that from cereal root eelworm, might have been overcome by substituting flax or linseed (*Linum*) for cereals and the third by processing larger soil samples but the second could only be overcome by greatly increasing the number of plots to give more replication.

The results of the experiment up to the fourth year are given in Fig. 1. Those for blocks Nos. 1 and 3 present a reasonable picture of population changes from 1951 to 1955. Those for block No. 2. suggests that sugar beet was not able to maintain the high 'ceiling' level of 1953 in the following years. In 1954, the eelworm population of the beet plots in this block (No. 2) appears to have fallen more rapidly than in plots growing non-host crops. Active reduction of very high populations by growing beet has previously been observed (Jones 1956) and more evidence for its occurrence is presented later (page 263).

An Experiment to Test the Effects of Three Host Plants grown at Four Population Levels.

In 1956 the Oxlode microplots were used for an experiment to test the effects of sugar beet, cress (*Lepidium sativum* L.) and charlock (*Sinapis arvensis* L.) on the beet eelworm population when grown at four different plant population levels. These hosts were chosen because they are considered to represent three levels of host efficiency, cress being the most efficient, beet less so and charlock rather inefficient (Jones 1956). The plant populations employed were 1, 4, 16 and 64 per plot which are equivalent to 6,000, 24,000, 96,000, and 384,000/acre (15,000, 60,000, 240,000, and 960,000/hectare). Previous work (Jones 1956) in the same soil had suggested that the final eelworm population was independent of plant density. Since the vegetative period of sugar beet (April-November) is longer than that of cress and charlock (April-July), a second series of sugar beet plots was included, the plants being lifted in July to give the same period of growth as the cress and charlock (i.e. April-July). The plots were

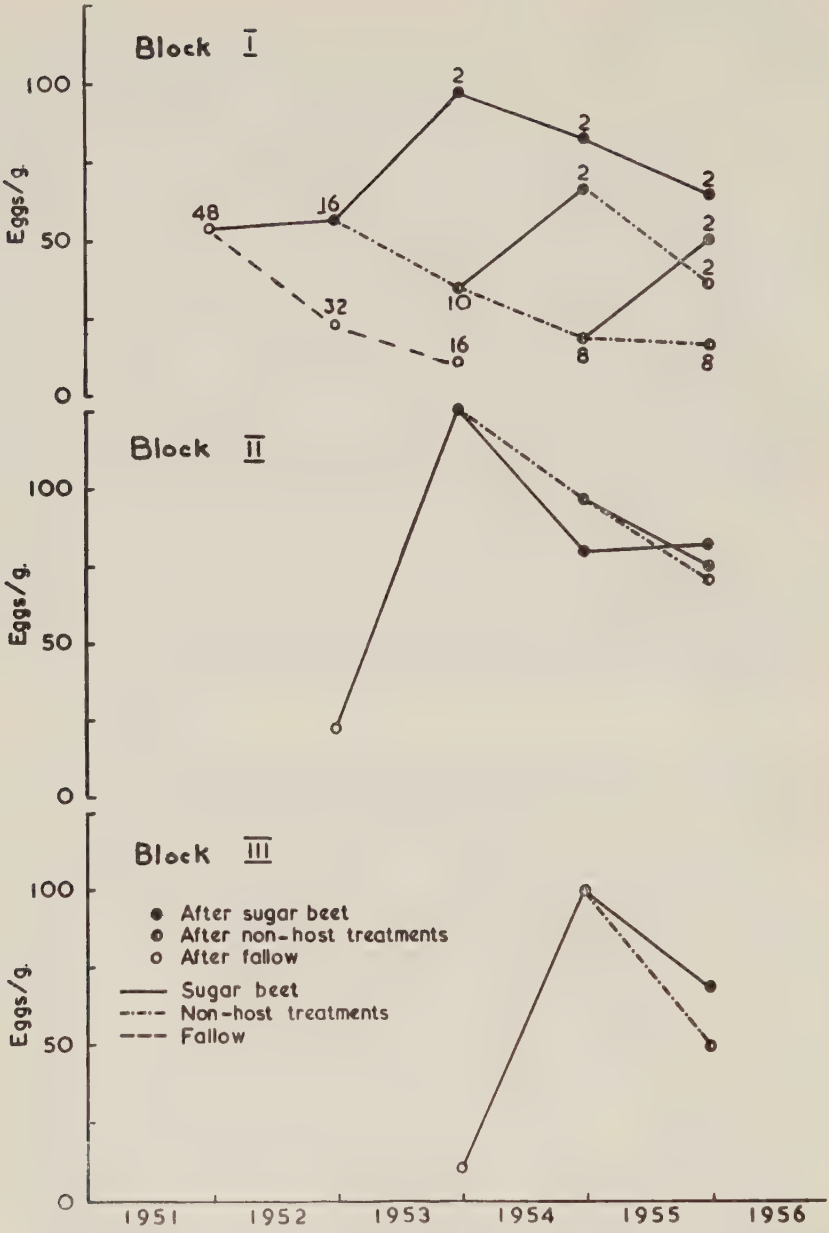

Fig. 1. Four years' results from a rotational experiment. The numbers in Block No. 1. indicate the numbers of plots upon which each population estimate is based, i.e. its 'weight'.

divided into three 'levels' of initial populations and the treatments randomized within these 'levels' which acted as blocks. Altogether the experiment occupied 48 plots. In the statistical treatment of the results, the final cyst and egg counts were analyzed in square root transformation, the standard procedure for counts subject to a Poisson error.

Results are presented in Table III. In the cyst populations there were no significant differences between the effects of sugar beet, cress or charlock, or between beet grown during the period April-July compared with beet grown during April-November. The difference between cress and charlock, however, approached significance. Plant numbers had a significant effect, 1 and 4 plants/plot giving lower cyst populations than 16 and 64/plot, but the differences were not great. There was a general fall in egg populations from an average of 242 eggs/g. before sowing to 151 eggs/g. after harvesting. Charlock gave a significantly lower figure than the other plants which were not significantly different amongst themselves. There were no significant differences in egg counts due to plant populations. Comparison of sugar beet and cress when grown at their respective plant population levels as in field crops, i.e. beet at 4 plants/plot and cress at 64 plants/plot, shows that cress gave significantly higher cyst counts but no significant increase in eggs. Perhaps the most interesting observations afforded by the experiment are the relatively small differences created by widely divergent plant populations and by sugar beet grown for "short" and "long" periods allowing time for one and two generations respectively.

The Effects of a Range of Host Plants on the Beet Eelworm Population of two types of Sandy Loam.

The Spalding and Cantley soils fall into the same textural class, fine sand, but differ considerably (Table II). The Spalding soil contains a very high proportion of fine sand (74 %) bordering on silt while the Cantley soil contains an appreciable fraction of coarse sand (29 %). In 1955, the Spalding and Cantley microplots were sown with a range of Chenopodiaceous and Cruciferous host plants, replication being three-fold for the Cantley plots and two-fold for the Spalding plots. In both cases four plots were left fallow as controls. To avoid the effects of different plant populations, four plants of each crop were grown per plot. The growing periods of the plants

TABLE III

The effects of host plants and plant populations on the final beet eelworm population of Oxlode soil

Population Plants/plot	Sugar Beet April-July	Sugar Beet April-November	Cress April-July	Charlock April-July	Means for population levels	
			$\sqrt{Cysts/200\ g.\ soil}$			
			*(± 3.81)			
1	23.6	25.5	24.4	24.4	24.5	
4	24.4	25.3	25.5	23.7	24.7	(± 1.91)
16	28.0	27.3	29.7	26.7	27.9	
64	29.4	28.9	29.6	27.0	28.7	
Means for host plants	26.3	26.8	27.3	25.5	26.5	
			(± 1.91)			
			Cysts/200 g. soil			
			Treatment means (calculated from transformed values)			
1	557	650	595	595	600	
4	595	640	650	562	610	
16	784	745	882	713	778	
64	864	835	876	729	824	
Means for host plants	692	718	745	650	702	
			$\sqrt{Eggs/g.\ soil}$			
			(± 3.15)			
1	12.6	12.6	10.7	12.0	12.0	
4	12.1	13.9	11.8	9.6	11.9	(± 1.56)
16	12.6	14.6	12.2	8.6	12.0	
64	11.9	14.2	15.0	11.6	13.2	
Means for host plants	12.3	13.8	12.4	10.5	12.3	
			(± 1.56)			
			Eggs/g. soil			
			Treatment means (calculated from transformed values)			
1	159	159	114	144	144	
4	146	193	139	92	142	
16	159	213	149	74	144	
64	142	202	225	135	174	
Means for host plants	151	190	154	110	151	

* The values in brackets are the least significant differences. Those above the figures refer to comparisons between any two of the 16 treatment means below them. Similarly, those below the figures refer to the four "host plant" means and those to the right to the four "plant population" means.

were variable, however, spinach (*Spinacia oleracea* L.), coleseed (*Brassica napus* L.), turnip (*Brassica rapa* L.), charlock (*Sinapis arvensis* L.), mustard (*S. alba* L.), radish (*Rhaphanus sativus* L.) and cress (*Lepidium sativum* L.) grew throughout the period April-early August, while the remainder except for sowbane (*Chenopodium hybridum* L.) grew from April to October. The sowbane, although

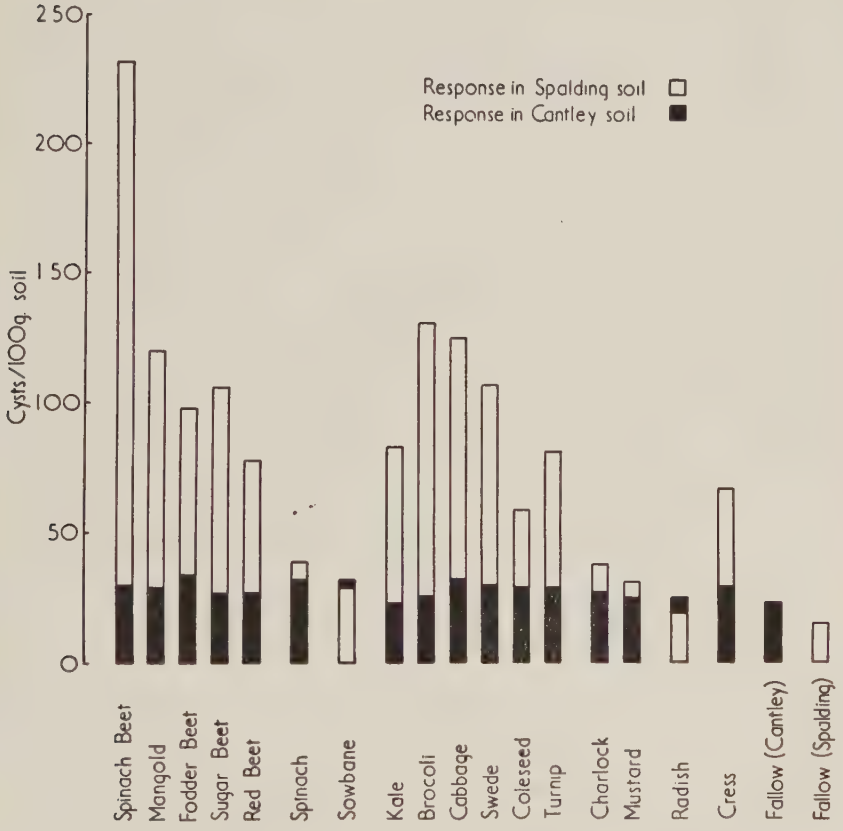

Fig. 2. The population responses in terms of cysts/100 g., soil from a range of host plants grown at 4 plants/plot in two different fine sandy loams.

sown in April, did not germinate until late June and was harvested late in August.

Results are given in Figs. 2 and 3. Because of previous uniform treatment of the plots the same picture is presented by cyst and egg counts. The results of the Spalding plots indicate the relative efficiency

of the various hosts compared on a plant-for-plant basis. The results are much the same as those previously obtained in Chatteris soil working at an initial population of 56 eggs/g. soil (40 hatchable larvae/g.) except that the differences between Cruciferae and Chenopodiaceae are less marked. The higher levels given by Cruciferous seed crops in the Chatteris soil may perhaps have been due to the higher

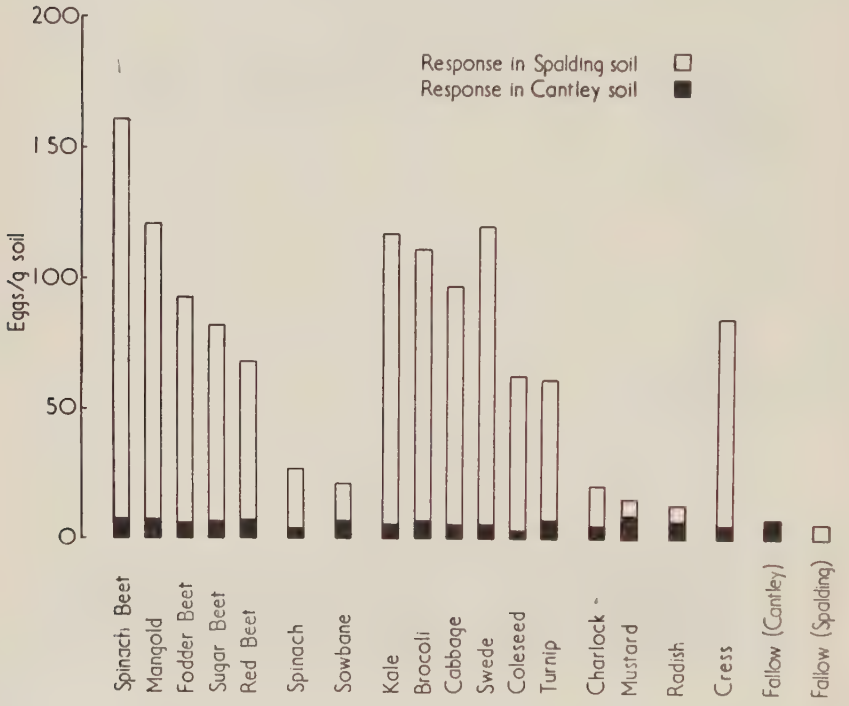

Fig. 3. The population responses in terms of eggs/g. soil from a range of host plants grown at 4 plants/plot in two different fine sandy loams.

plant populations employed, for in that experiment the hosts were grown as in agricultural practice and comparison was on a crop-for-crop and not plant-for-plant basis.

The results from the Cantley soil (Fig. 2 and 3) were once again anomalous, no detectable increase in cyst or egg populations having occurred. All plots, regardless of the host plants grown, contained cyst and egg populations not significantly different from the fallow control plots. Because of the low level of the Cantley populations, cysts were difficult to identify and the total cyst counts are plotted in Fig. 2.

Microscopic examination of samples of cysts showed that the plots contained approximately 15 % *Heterodera schachtii*, 44 % *H. major*, 14 % of cysts tentatively ascribed to *H. cruciferae* and 27 % indeterminate. Total eggs are plotted in Fig. 3; about one-third of these came definitely from *H. schachtii* cysts. Evidently the *H. cruciferae* fraction of the population had behaved like the *H. schachtii* fraction

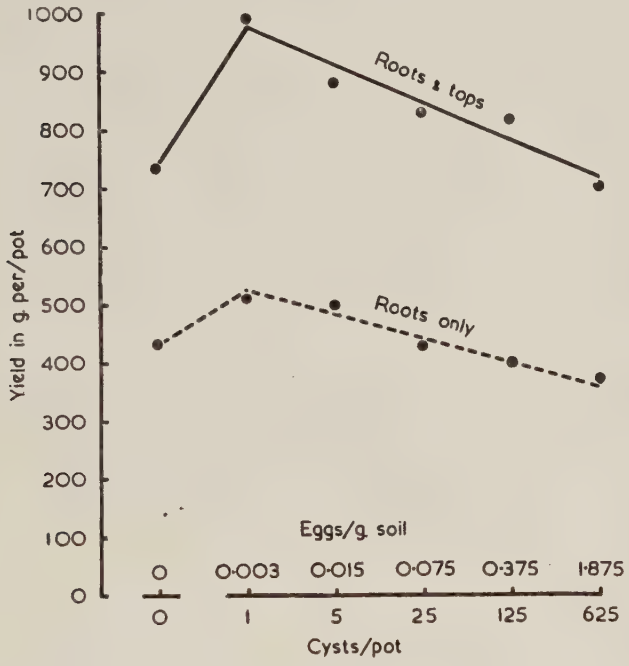

Fig. 4. Results from an experiment in large glazed pots: the relationship of yield to inoculation level. For convenience, the yields of the control pots, to which no cysts were added, are plotted at a false origin.

and had not increased on the Cruciferous plants grown. Hatching tests done in the winter of 1956 suggested that there was no appreciable *H. cruciferae* population in the soil. Since the Spalding and Cantley plots lay side by side and growth of the plants in both was not noticeably different, the striking differences in response between their respective beet eelworm populations were evidently due to some inherent properties of the two types of soil.

A Pot Experiment to test the effect of low inoculation levels on yield.

In the field and in small plots low soil population levels cannot be measured accurately (Jones 1955). To test low levels of infection on

yield, therefore, a pot experiment was set up. Cysts derived from Chatteris soil were added in a logarithmic series (1, 5, 25, 125 and 625, and controls without cysts) to glazed pots each holding 10 kg. of a mixture of 4 : 1 sterilized potting loam and sand. Replication was fivefold. The cysts were floated in bulk and added to the pots in the

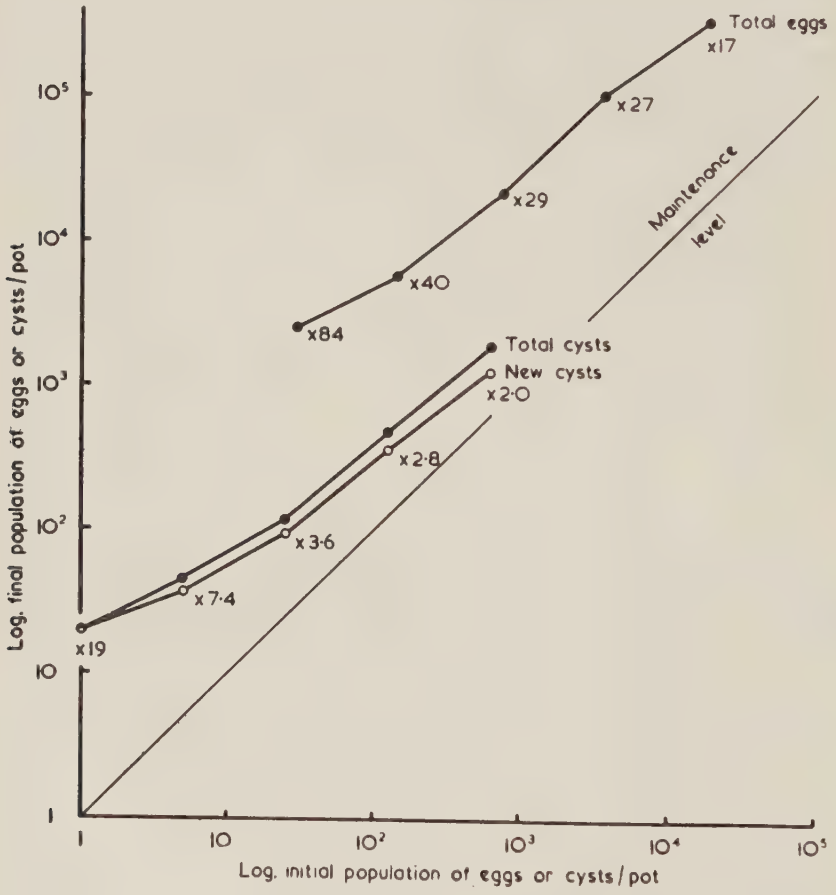

Fig. 5. Results from an experiment in large glazed pots: the relation between initial and final eelworm populations. New cysts: derived from total cysts by subtraction of the number introduced by inoculation.

moist condition. They contained an average of 30 eggs/cyst. Appropriate batches were scattered in a depression in the upper layers of pot soil, covered and stirred into the top 6 inches. This procedure had the effect of increasing the effective concentration in the neigh-

bourhood of the plant by a factor of 3, leaving the lower layers free until invaded by larvae. After inoculation, the pots were randomized on an outdoor site and a small beet seedling transplanted into each of them on May 25th. Good growth took place and harvesting was completed on October 12th, giving a rather short growing season.

The effects of inoculation on yield are shown in Fig. 4. The lowest level, 1 cyst/pot, increased total yield significantly and thereafter there was a significant inverse linear regression of total yield upon log. inoculation level. Root yields behaved similarly. The final eelworm populations were disappointingly low (Fig. 5) possibly because the short growing period and the cool weather from July onwards did not allow completion of a second generation. No large increases comparable with those reported by GOFFART (1952) were observed and there was no evidence of a 'ceiling' effect (JONES 1956). The maximum rate of increase was 19 times for cysts and 84 times for eggs at the lowest inoculation level. The rates of increase fell off progressively for higher inoculation levels. At the lower inoculation levels, 1 and 5 cysts/pot, only approximate egg populations and increase rates are plotted because the average egg content of the cysts varies widely. This difficulty is inherent in inoculation experiments with cysts where low numbers are used whatever the average cyst content.

DISCUSSION

The process of development in *Heterodera* spp. may be divided into a number of separate stages:

(1) Hatching
(2) Emergence from the cyst
(3) Movement of the larva through the soil
(4) Invasion of the plant rootlet
(5) Early development
(6) Ovulation
(7) Maturation of eggs and encystment

Stages (1) and (4) are governed largely by the soil environment, especially its physical structure and water content (WALLACE 1955, 1956a, 1956b). Some influence, however, is exerted by the plant through root diffusates and also probably by other exuded chemicals which provide directional stimuli to the migrating larvae. After invasion, development and ovulation are probably dependent on the environment supplied by the plant and to a considerable degree independent of soil conditions although the plant root is itself influenced

by these. Maturation of eggs may be independent of plant and soil, provided there is sufficient moisture.

In the rotational experiment and in previous experiments (JONES 1956), increase in the beet eelworm population has occurred in the Chatteris soil. For two consecutive years (Table I) an increase occurred in Spalding soil, a decrease in Oxlode soil and no detectable change in the Cantley soil. Since macroclimatic conditions in the microplots were the same for all soils and in the absence of information concerning nematode enemies, it is assumed that these differences in behaviour between the beet eelworm populations of the soils are due to differences in the soil structure/moisture relationships acting principally upon stages (1) to (4) in the development of *H. schachtii*.

In the Fens, *in situ*, the Oxlode soil enjoys a high water table and there, in 1953, produced a population of 480 eggs/g. on sugar beet. In the microplots, in a drier situation, the level has fallen for two successive years under cultivation of sugar beet and other efficient host plants. Where, for whatever cause, soil conditions permit the completion of stages (1) and (2) but inhibit subsequent stages, the effect of host plants is mainly exerted by root diffusate action in emptying cysts of larvae that are in a responsive state. Provided sufficient diffusate is produced, the result is likely to be much the same for different hosts and for different plant population levels. This may be the explanation of the relative insensitivity of Oxlode soil to host plants shown in Table 3 and in JONES (1956) Table III. It must be admitted, however, that the growth of plants in this soil is less vigorous than it would be *in situ* so that the somewhat reduced root growth may be partly responsible. But this factor does not appear to enter into the striking differences in soil population response shown by the Spalding and Cantley soils. In both plant growth was excellent. In the Spalding soil larvae succeeded in invading roots and in producing increase. In the Cantley soil probably invasion did not occur. Had it done so there seems no valid reason why invaded larvae should have failed to reach maturity.

The relationship between yield and log. inoculum level in the pot experiment agrees with previous results from beet eelworm in microplots (JONES 1956) and with results obtained by LOWNSBERY & PETERS (1955) for *H. tabacum* Lownsbery & Lownsbery. A similar relationship has also been reported for *H. rostochiensis* by PETERS (1954) and for *H. cruciferae* by DONCASTER (*in litt.*). In these experiments, evidence of a stimulative action on growth by low inoculum

levels was obtained but did not reach significance. In the pot experiment with beet eelworm herein described, much lower inoculum levels were used than those reported by PETERS or LOWNSBERY & PETERS, and a significant increase in yield was obtained at the lowest inoculum level. Stimulation of growth by low inoculum levels therefore appears to be a general phenomenon the reasons for which are at present unknown.

ACKNOWLEDGEMENTS

Thanks are due to Elizabeth Widdowson for help with cyst and egg counts, to J. H. A. Dunwoody for help with statistics and to J. J. Hesling for help with cyst identifications. I am also indebted to F. Moriarty for hatching tests made on cysts from the Cantley plots.

ZUSAMMENFASSUNG

Bei früheren Untersuchungen über die Populationsdynamik des Rübennematoden (*Heterodera schachtii* Schm.) wurden Feldstücke von 2 acre Grösse nach einem festgelegten Gittersystem geprüft. Später wurde eine Mikroparzellen-Technik entwickelt, um die veränderlichen Faktoren mehr in die Hand zu bekommen. Anlage und Ergebnisse eines Fruchtfolgeversuchs auf Mikroparzellen werden mitgeteilt. Die Populationsveränderungen erfolgten wie erwartet, mit Ausnahme eines Falles, bei dem eine deutliche Verminderung einer hohen Population nach dem Anbau von Rüben eintrat. Schwierigkeiten in der Trennung von Rüben- und Getreidenematodencysten konnten durch den Anbau von Flachs anstelle von Weizen und Gerste vermieden werden. Ungenauigkeiten bei der Berechnung niedriger Populationen am Ende eines drei- bis vierjährigen Anbaues waren nur durch stark vermehrte Wiederholungen zu beseitigen.

In einem Mikroparzellenversuch auf einem humosen Tonboden mit hoher Anfangspopulation wurde der Einfluss von Zuckerrübe, Kresse und Ackersenf bei unterschiedlich starker Pflanzenpopulation verglichen. Trotz erheblicher Abweichungen in der Pflanzenzahl ergab sich nur ein geringer Unterschied in der endgültigen Älchenpopulation. Durchschnittlich trat ein Rückgang von 242 Eier je g. Boden auf 151 Eier ein.

Eine Reihe Wirtspflanzen wuchs bei derselben Pflanzenpopulationshöhe auf Mikroparzellen, die zwei Arten feinen sandigen Lehms enthielt (infiziert mit Nematodencysten aus dem vorhergehenden humosen Tonboden). In dem einen Boden trat ein beträchtliches Ansteigen, in dem anderen keine Zunahme der Cystenmenge ein. Da das Pflanzenwachstum in beiden Böden fast gleich war, scheinen diese Unterschiede mit Abweichungen in der physikalischen Struktur und mit anderen Eigenschaften der Böden in Beziehung zu stehen.

Die möglichen Ursachen für das Absinken einer Älchenpopulation in humosen Tonboden und für des Ausbleiben einer Steigerung in feinem sandigem Lehm werden erörtert. Physikalische Bodenfaktoren wirken sich wahrscheinlich auf das Schlüpfen, die Bewegung und den Befall der Pflanzenwurzeln unmittelbar aus, während die späteren Stadien in der Entwicklung innerhalb der Pflanze nur mittelbar durch diese beeinflusst werden.

Mitgeteilt werden ferner die Ergebnisse eines Versuchs in grossen glasierten Töpfen. Niedrige Infektionen mit Rübennematoden schienen das Wachstum der Rübe zu stimulieren. Bei höherer Infektion war der Ertrag umgekehrt proportional dem log. der Infektion. Der Populationsanstieg war niedriger als bei den von GOFFART (1952) beschriebenen Versuchen, wahrscheinlich, weil die kurze Wachstumsperiode die Entwicklung einer zweiten Generation nicht erlaubte.

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ERRATUM

Nematologica II: 202-214 (P. W. MURPHY & C. C. DONCASTER).

1. Page 212, Table II, bottom line. The grand total 1,216 refers to *H. cruciferae,* "undamaged", damaged by "cause unknown" and "damaged by Collembola". — 6,9 *should read* 6.9

2. Plate XV, Fig. 6. "performations" *should read* "perforations".

C.C. DONCASTER

Nematologica II (1957) : 273-276. — Leiden, E. J. Brill

A NOTE ON NEMATODE PARASITES OF RED ANTHURIUM (*ANTHURIUM ANDRAEANUM* LIND.), WITH A DESCRIPTION OF *ROTYLENCHUS BOOCKI* N. SP. [1])

BY

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Two samples of diseased roots from stunted plants of red anthurium (*Anthurium andraeanum* Lind.) were attacked by *Meloidogyne inornata* Lordello, 1956, and an undescribed form of *Rotylenchus* Filipjev, 1934. The second sample was also attacked by *Helicotylenchus nannus* Steiner, 1945.

On anthurium, *M. inornata* produces very small galls which may easily be overlooked; indeed, the diameter of the galls is only a little greater than that of the root itself. The tissues around the point of invasion undergo necrosis, producing characteristic dark areas from which the cortex of the root is easily detached.

A female *Helicotylenchus nannus* had the following measurements (in μ): length, 624.6; width, 26.0; stylet, 27.5; distance between the orifice of the dorsal oesophageal gland and the stylet knobs, 13.7; tail length, 15.3; a = 24.7; b = 7.0; c = 42.0; V = 61 %.

A description of *Rotylenchus boocki* n.sp. is given below. Of the three species of nematodes found on anthurium, *M. inornata* appears to be the most important.

Description of *Rotylenchus boocki* n.sp. (Fig. 1)

♀: (measurements, in μ): total length, 850.0-863.0; width, 38.2; stylet, 29.0; distance between the orifice of dorsal oesophageal gland and the stylet knobs, 9.2; tail, 13.8-15.3; a = 22.2-22.5; b = 7.0-7.6; c = 61.5; V = 60.9-61.4 %.

♂: unknown.

Body of fixed female specimens forming a more or less open spiral, slightly tapering caudad from the vulva and more abruptly cephalad starting at the region of the oesophago-intestinal junction.

Cuticle annulated; annules 1.7 μ apart. Annulation on tail following

1) Received for publication: March 25, 1957.

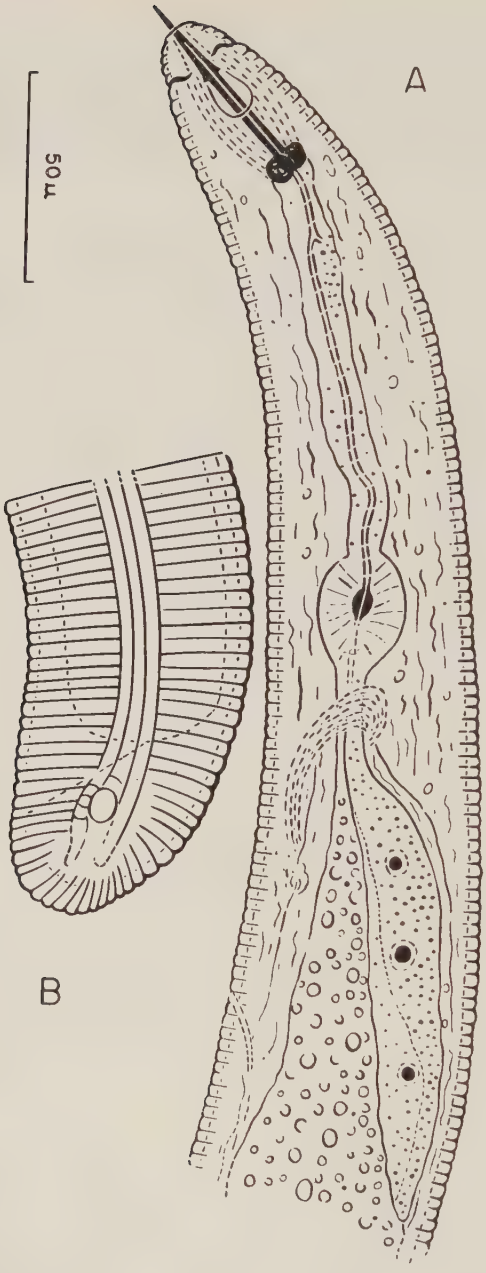

Fig. 1. *Rotylenchus boocki* n. sp. A: anterior region of female; B: tail end.

contour of end. The lateral fields begin about 50 μ from head and extend to end of tail, being aerolated in the oesophageal region. The fields are first made up of three incisures. But, at latitude of isthmus the middle incisure bifurcates and the fields continue to the tail with four lines. The area resulting from the bifurcation of the middle incisure is not aerolated anywhere.

Head well set off from neck, with five annules. Amphids not seen. Stylet strong, 29 μ long, with somewhat spherical basal knobs. Oesophagus typical. The distance between the orifice of the dorsal gland and the stylet knobs being 9.2 μ, is a little shorter than one-third of the total stylet length. The oesophageal glands are quite long and overlap the beginning of intestine.

Intestinal cells filled with globular reserve material, that makes accurate study of the sexual organs very difficult. The intestine ends in a short rectum.

Excretory pore located at the latitude of posterior oesophageal gland.

Sexual apparatus amphidelphic, with anterior, as well as posterior branches to the right of the intestine, each branch about 30 % of total body length. Vulva a transverse slit at 522-526 μ from head. The vulval opening represents a little more than one-half of body width. Vagina with thickened walls, leading straight inward for one-third of body width. No eggs seen in the uterus, which probably can hold only one egg at a time. Ovaries outstretched; oocytes disposed in tandem arrangement.

Tail end broad. Phasmids large (*scutellum*), their outlines filling up the space between the inner incisures of the lateral fields, opposite to each other, located in front of anus. A few striae run from the phasmids across the ventral band of the lateral fields, reaching the ventral incisures.

Diagnosis — *Rotylenchus* with the above characters. The species resembling *R. boocki* n. sp. most closely is *R. brachyurus* Steiner, 1938, from which it differs in having: a) shorter oesophagus (b = 7.0-7.6: b = 5.6-6.4); and, b) longer distance between the orifice of the dorsal gland and the stylet knobs. In addition, in *R. boocki* tail, a few striae joining the edges of the phasmid to the ventral incisures of the lateral fields are seen. Such striae are absent in *R. brachyurus*. The main difference between the two species, however, is the distance from the opening of the oesophageal gland to the stylet knobs. As clearly figured by Steiner (1938), this distance in *R. brachyurus* is one-ninth of the stylet length, while in *R. boocki* it is about one-

third. *R. boocki* is intermediate in this character between *Rotylenchus* and *Helicotylenchus* species, but the form of the phasmids places it in *Rotylenchus* (GOLDEN, 1956).

Type locality: S. Paulo, Brazil.

The type specimens are in the writer's collection.

The new *Rotylenchus* described represents the third spiral nematode found in the State of S. Paulo. The previously recorded forms are *R. melancholicus* Lordello, 1955 and *H. nannus* Steiner, 1945, (LORDELLO & ZAMITH, 1956).

I wish to thank Mr. R. CESNIK for the samples of red anthurium on which the above nematodes were found. The specific name in *R. boocki* is given in honour of Dr. O. J. BOOCK, Instituto Agronômico, Campinas, for his encouragement of Plant Nematology in Brazil.

RESUMO

Êste trabalho constitui uma contribuição ao conhecimento dos nematódeos que parasitam o antúrio vermelho no Brasil. O estudo de material coligido pelo Snr. R. CESNIK, em S. Paulo, revelou a presença de três espécies atacando o sistema radicular, a saber: *Meloidogyne inornata* Lordello, 1956; *Helicotylenchus nannus* Steiner, 1945; e *Rotylenchus boocki* n. sp., das quais a primeira parece ser a mais nociva.

Em consequência do parasitismo, as plantas apresentam desenvolvimento precário, não florescem e emitem fôlhas pequenas, anormais. Nas raízes, notam-se pequenas galhas, necroses generalizadas e descolamento do córtex.

São registradas observações acêrca dos dois parasitos conhecidos e feita a descrição da nova espécie.

R. boocki é um *Rotylenchus* muito próximo de *R. brachyurus* Steiner, 1938, do qual difere: a) pelo esôfago mais curto (b = 7.0-7.6: b = 5.6-6.4); e, b) pela maior distância entre o orifício onde vem se abrir, no canal do esôfago, o conduto da glândula dorsal e os bulbos do estilete. Em adição, na região caudal de *R. boocki* aparecem algumas estrias unindo os limites dos *phasmids* às incisuras ventrais dos campos laterais, estrias esas inexistentes em *R. brachyurus*.

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Nematologica II (1957) : 277-284. — Leiden, E. J. Brill

SOME EXPERIMENTS ON THE VACUUM DISTILLATION OF POTATO ROOT DIFFUSATE [1])

BY

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In a previous paper (FENWICK & WIDDOWSON, in press), methods were described for collecting and storing potato root diffusate. Storage at 3-4° C is satisfactory for up to two years but lack of space frequently limits the volume which can be stored at any given time. Attempts were therefore made to reduce this volume by vacuum distillation. Previous experiments in this connection have been described by HAGUE (1954) who found that vacuum distillation at 5° C at 1 mm. of mercury could be carried out with a reduction to 1-4 % of the original volume and a loss of only 5-10 % of the active principle. Subsequent freeze drying resulted in higher concentration with a further loss of about 30 %. HAGUE did not describe his apparatus but the low temperatures and pressures involved suggest rather elaborate equipment. It would be convenient to carry out the distillation at a higher temperature with simple equipment and the present paper is concerned with an investigation into this possibility.

Stability of Potato Root Diffusate to Temperature

Experiments were carried out on the stability of potato root diffusate at different temperatures. Samples were maintained at 30, 40, 50 and 60° C for 2, 4, 8, 16, 32 and 64 hours after which they were assayed by the methods of FENWICK & WIDDOWSON (in press). Replication was five-fold; untreated samples gave L.A. values of approximately 2.8 and larval emergence in these averaged approximately 140 larvae per cyst over three weeks corresponding to over 80 % of total contents. The results of these assays are set out in Fig. 1. Fall in activity at 30 and 40° C after 8 hours was very slight; the loss at 50 and 60° C was greater, the L.A. value falling by about 0.5. 16 hours at 30 and 40° C resulted in a fall of about 1.0 L.A. units corresponding to a loss of 90 % of the active principle. Repetition of

1) Received for publication: April 11, 1957.

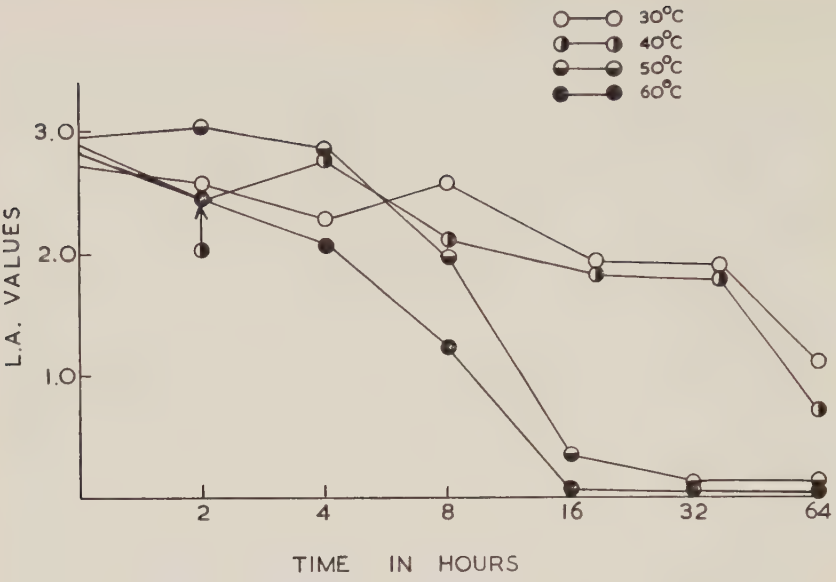

Fig. 1. Effect of temperature on the stability of Potato Root Diffusate.

this experiment with other samples of diffusate gave similar results, a typical set of data being given in Table I. A large number of tests

TABLE I

L.A. values of samples of potato-root diffusate exposed for different times to 30°, 40°, 50° and 60°.

Time in hours	Temperature			
	30°C	40°C	50°C	60°C
0	1.75	1.90	1.75	1.69
2	1.83	1.62	1.92	1.80
3	1.88	1.72	1.79	2.05
4	1.98	1.84	2.05	1.64
8	1.98	1.92	1.50	0.76
16	1.92	1.70	0.65	0.28
32	1.58	0.76	0.28	0.15
64	1.37	0.76	0.23	0.19

(Note: A fall of 1 L.A. unit indicates a tenfold decrease in activity.)

showed that up to 8 hours exposure to 30 or 40° C resulted in only a slight loss of activity; the average decrease in L.A. value for eight hours at 40° C was about 0 15 L.A. units corresponding to a 30 %

loss in active principle. No detectable loss in activity resulted from 40° C for shorter times or 30° C for up to eight hours.

A further test sought to investigate in more detail the effect of short exposures to 50° C: the results of tests on two samples of diffusate are set out in Fig. 2. Up to three hours exposure resulted in negligible loss but longer times resulted in a rapid loss of activity. Repetition of this experiment yielded similar results.

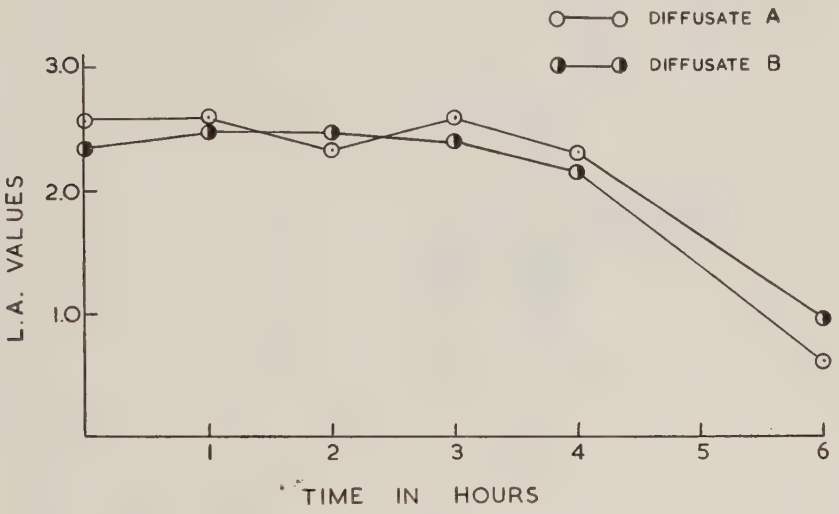

Fig. 2. The effect of short exposures to 50° C on the activity of Potato Root Diffusate.

The results indicate that vacuum distillation might be practical at higher temperatures than were used by HAGUE. If 40° C were not exceeded, efficient concentration should be possible if it took no longer than four hours: at 50° C concentration should not occupy more than two hours. Under these conditions little loss of active principle should occur.

Experiments on Vacuum Distillation

The apparatus used is shown in Fig. 3. A bolthead flask of 1 litre capacity, filled with a four-hole bung rests on the water bath B, heated by the burner C; one hole of the bung is fitted with a thermometer D; another with a tube E, the lower end of which is drawn to a fine capillary extending to the bottom of the flask; a third hole carries a tube connected by rubber tubing and a screw clip (F) to a two litre reservoir of root diffusate: the remaining hole is fitted with the

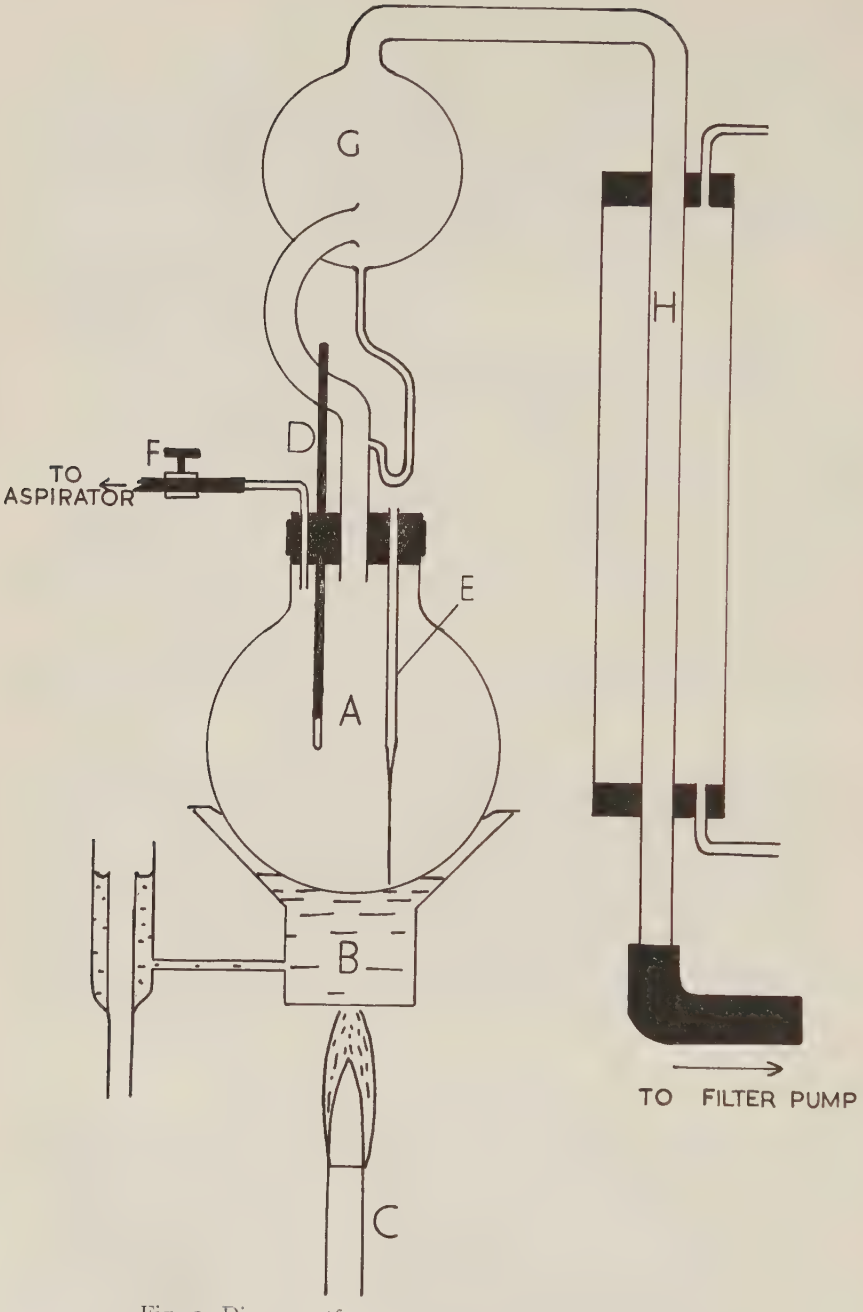

Fig. 3. Diagram of apparatus for vacuum distillation.

cyclone head G which is connected by wide bore tubing to the aluminium condenser H. The lower end of the latter is connected by wide bore pressure tubing to a filter pump. About 500 ml. of fluid is run into the flask A and the clip F closed; the pump is turned on and the burner under the water bath lit; the fluid in A boils at 30-35° C at a pressure of a few mms. of mercury. The screw clip F can be adjusted so that entry into A just balances the loss by evaporation. A fine current of air drawn through the fluid by the capillary ensures smooth boiling without bumping. Normally two litres of diffusate can be reduced to 1/10 or 1/20 of its original volume in about two and half hours. A number of such distillations were carried out. Assay curves on samples taken before and after concentration are given in Fig. 4;

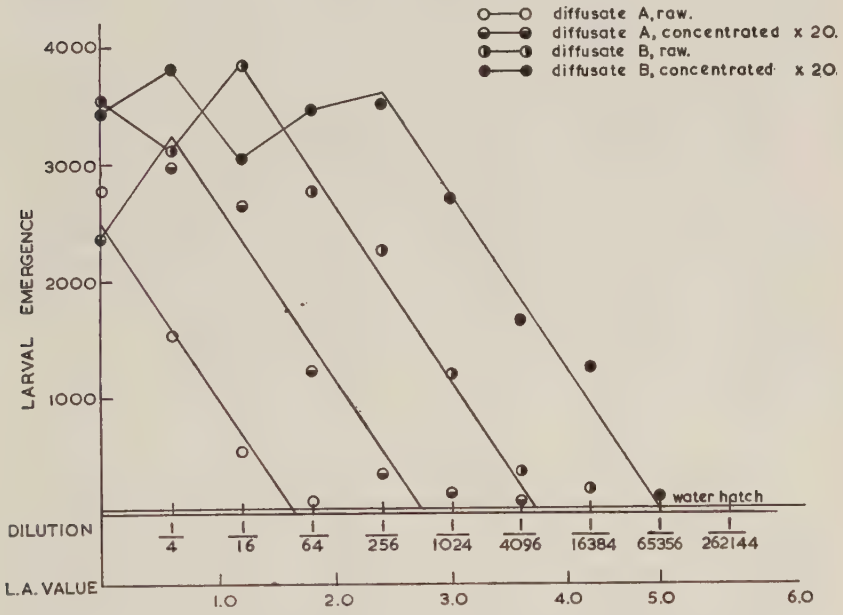

Fig. 4. Assay curves on samples of diffusate before and after vacuum distillation.

the concentration was taken to 1/20 the original volume. The straight line sections of the curves were obtained by calculation, a common slope being computed for all four lines. Diffusate A had an L.A. value of 1·62 before and 2·72 after distillation corresponding to a concentration factor of × 12·5. Comparable figures for diffusate B were 3·72 and 4·80 corresponding to a concentration factor of 12·0. The efficiency of the method would therefore appear to be about 60 %.

Although this level of efficiency is lower than that claimed by HAGUE it is still sufficiently high to render the process practical in view of the decreased bulk involved and the consequent economy in storage space.

A number of other samples of root diffusate were distilled and the relevant data before and after concentration are given in Table II. It

TABLE II

Results of vacuum distillation of potato-root diffusate.

Sample	Concentration by distillation	L.A. before.	L.A. after	Concentration of factor achieved in final product	% efficiency
1	x10	3.4	4.4	x10	100
2	x10	3.4	4.3	x 8	80
3	x10	3.2	4.0	x 6	60
4	x10	3.3	3.4	x12	120?
5	x10	3.2	4.0	x 6	60
6	x10	3.3	4.2	x 8	80
7	x10	3.2	4.2	x10	100
8	x10	1.9	2.8	x 8	80
9	x10	2.8	3.6	x 6	60
10	x10	2.1	2.2	x12	120?
11	x10	2.4	3.3	x 8	80
12	x10	2.4	3.5	x12	120?
13	x10	2.4	3.2	x 6	60
14	x10	2.6	3.6	x10	100
15	x10	2.4	3.2	x 6	60
16	x10	2.5	3.5	x10	100
17	x20	2.3	3.7	x25	120?
18	x20	3.1	3.8	x 5	25
19	x20	1.9	3.2	x20	100
20	x20	2.1	3.4	x20	100
21	x20	2.4	3.9	x31	150?
22	x20	2.4	3.6	x16	80
23	x20	2.3	3.7	x25	120?
24	x20	2.8	4.1	x20	100

will be seen that in general very high levels of efficiency were obtained — the standard error applicable to the assays on which this table is based was in the neighbourhood of 20 % so that it is not surprising that some estimates of efficiency were greater than 100 %. The general picture would suggest that usually the level of efficiency attained was in the neighbourhood of 80 %. These results confirm

the view that vacuum distillation is a practical method of reducing the bulk of potato root diffusate for storage.

Further observations were made on the effect of evaporation of potato root diffusate in bulk. These observations are the by-products of monitoring assays on samples produced at the Department of Horticulture of the University of Reading. This work, carried out under the auspices of the Agricultural Research Council was part of a tripartite scheme which involved the Chemical Laboratory, University of Cambridge, the Department of Horticulture, University of Reading and the Nematology Department, Rothamsted Experimental Station. These samples were distilled in two stages: the first was done in a

TABLE III

Efficiency of vacuum distillation of a large quantity of diffusate.

L.A. raw diffusate, A	L.A. after first concentration, B	Increase of concentration B over A		Efficiency of first concentration	L.A. after second concentration, C	Increase of concentration C over B		Efficiency of second concentration	Increase of concentration C over A		Overall efficiency
		Theor.	Actual			Theor.	Actual		Theor.	Actual	
1.2	2.5	×15.5	×20	124%	3.2	×6.5	×5.0	77%	100	100	100%
2.6	3.7	×27	×12.6	47%	—	—	—	—	—	—	—
1.7	2.7	×14	×10.0	72%	3.2	×5.5	×3.16	58%	×80	×32	40%
1.7	2.6	×14	× 8	57%	3.0	×5.5	×2.5	45%	× 8?	×20	25%
1.9	3.3	×14	×25	?	3.8	×5.5	×3	54%	×80	×79	99%
2.4	3.3	× 8	×25	32%	4.2	×5.5	×8	?	×80	×63	79%
2.1	3.4	×14	×20	71%	—	—	—	—	—	—	—
1.9	2.5	×14	× 4	28%	2.7	×1.6	×8	20%	×80	× 6	8%

commercial milk evaporator working at 43.5-48.5° C under 710 mms. vacuum resulting in a concentration of about 15 times: the second was done in an all glass apparatus and was continued until the total volume was reduced to about 1/80 of the original. The results of a number of such evaporations are given in Table III and it will be seen that although the efficiency of the method was rather variable, very high levels were obtained on occasions. It is not possible to ascribe accurate levels of precision to the assays on which this table was based, all samples spent a variable time in the post and when the assays were carried out, only one dilution was tested: it is therefore very unlikely that the precision of estimating L.A. values was greater than 30 % and a value of 50 % might well be a better estimate.

It is therefore reasonable to conclude that vacuum distillation of potato root diffusate is a practical method of decreasing its volume for storage. Table II shows that for a decrease in volume to 10 % the loss of active principle is only about 10 % which makes the procedure well worth while.

ACKNOWLEDGEMENTS

The author's thanks are due to Professor R. H. Stoughton of the Department of Horticulture, University of Reading for arranging the bulk evaporation of potato root diffusates and for permitting the use of data resulting from them.

ZUSAMMENFASSUNG

Versuche wurden zur Klärung der Stabilität des Kartoffelwurzeldiffusats bei verschiedenen Temperaturen durchgeführt. Nach 8-stündiger Einwirkung von 30° oder 40° C oder nach 3-stündiger Einwirkung von 50° C trat nur ein unbedeutender Verlust der Aktivität ein. Destillation im Labor bei 30 bis 40° C führt zu einer Reduktion um 1/10 oder 1/20 des ursprünglichen Volumens mit einer Wirkung von 80 %. Auf kommerzieller Ebene an der Universität Reading durchgeführte Versuche werden mitgeteilt. Die Wirkung war unter diesen Bedingungen sehr verschieden, gelegentlich wurden hohe Werte erhalten. Die unterschiedliche Natur der Ergebnisse mag z.T. auf die Tatsache zurückzuführen sein, dass die geprüften Proben eine unterschiedliche Zeit vor dem Versuch unterwegs waren. Es wird geschlossen, dass Vacuum-Destillation eine praktische Methode zur Reduktion des Volumens für Aufbewahrungszwecke ist.

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ERRATUM

Nematologica II (1957): 242-249 (D. W. Fenwick).

The legends under Figs. 3 and 4 (p. 246 and 247) have been reversed. The graph above Fig. 3 should relate to fumigation at 20° C whilst the graph above Fig. 4 should relate to 15° C.

Nematologica II (1957) : 285-299. — Leiden, E. J. Brill

HETERODERA MAJOR O. SCHMIDT 1930 ON CEREALS — A POPULATION STUDY [1])

BY

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The increase of populations of certain *Heterodera* species, namely, *H. rostochiensis* (Wollenweber 1923) and *H. schachtii* (Schmidt 1871) has been shown to be dependent on the initial infestation, low infestations favouring high increases and vice versa (Chitwood and Feldmesser 1948, Fenwick and Reid 1953, Goffart 1952, Schmidt 1955). Chitwood and Feldmesser (1948), Peters (1954) and Lownsbery and Peters (1955) have observed that very low populations of *H. rostochiensis* and *H. tabacum* may increase host plant growth. These phenomena have not been investigated in detail in the case of *H. major,* nor has any plant which produces a root diffusate hatching stimulus for this species yet been demonstrated (Franklin 1951, Hesling 1957). It has been shown that the larvae of *H. major* enter oat roots in March, that the invasion of the roots reaches a peak in April and May and falls off in July (Schmidt 1931). Therefore, since *H. major* larvae continue to emerge over a period of several months during spring and early summer, there may be differences between this and other species of *Heterodera* regarding their effect on the host plant and the relationship between initial and final eelworm population, compared, for example, with *H. rostochiensis,* in which a very high proportion of the larvae may emerge from the cysts in four to five weeks, as a result of root diffusate stimulus (Fenwick and Reid 1953).

METHOD

In January 1955 *H. major* cysts were extracted by soil washing and elutriation (Hesling 1956) from partly dried soil of a field which had grown oats in 1954. These cysts were kept moist at approximately 2° C. In an attempt to reduce variability the cysts were graded by sieving

1) Received for publication: May 11, 1957.

and those cysts which did not pass through a mesh with apertures 0.556 mm. square were retained for the experiment. Each of these cysts was inspected using transmitted light, and those which appeared to be full were gently prodded with a blunt needle to check this. Cysts which could be seen to be wholly or partly empty, or which collapsed on prodding, were discarded. The cysts were sorted in this way in batches of about one hundred cysts and they were kept cold in iced water during the sorting process. In this way the stimulation of hatching by temperature rise was prevented. Selected cysts were quickly returned to store at 2° C. and, before their use as inoculum, they were mixed in suspension in ice-cold water and a batch of 179 was drawn off by pipette for the determination of the mean egg content per cyst. This was done by cutting open each cyst on a "perspex" slide with a sharpened Hagedorn needle, and washing the cysts and contents into a calibrated specimen tube 7 inches long and 1 inch in diameter. The resultant suspension was agitated for one minute by an electric stirrer provided with home-made rubber blades (Fidler 1956). From it samples were taken and the eggs and larvae counted, using a modified McMaster slide (Fenwick 1951).

The cereals were grown in pots of soil consisting of three parts unsterilised loam and one part coarse sand. Before use, more than two kilograms of the loam were examined for *Heterodera* cysts, but only *H. rostochiensis* cysts were found. The pots used had a top diameter of 19 cms, and each held about 2,370 grams of soil. The filled pots were placed outside until their inoculation with cysts so that the soil in them did not warm up prematurely.

The cysts were removed from cold storage and counted out into batches for inoculation at the levels shown in Table I.

Table I

H. major inoculation levels

Cysts per pot	0	1	2	4	8	16	32	64	128
Larvae per pot	0	445	890	1780	3560	7120	14240	28480	56960
Larvae per gram of soil	0	0.19	0.38	0.75	1.5	3.0	6.05	12.1	24.2

The soil in the pots was inoculated with cysts on the 14th March 1955. Small numbers of cysts were mixed into the soil with a trowel, but where there were eight or more cysts per pot the soil was tipped out on to a tray and the cysts mixed into the soil as thoroughly as possible. The soil plus cysts was then replaced in the pot and all pots

were placed in a gravel plunge. Replication was two fold, and on 30th March eight seeds were sown in each pot. The cereals used and their varieties were Oats — "S147", Wheat— "Atle", and Barley — "Herta". Germination was good, and from the beginning of June the height of each plant was measured at intervals of one week. This was done by placing a metre rule vertically against each plant and holding up the plant leaves or ears to their maximum height, which was then read off on the rule. When the grain was almost ripe the plants were cut down because of bird damage but the pots were left in the ground until late autumn, and the contents were then passed separately through a ¼ inch-mesh sieve. Straw and stones only were discarded and the soil from each pot was dried thoroughly and then washed through a Fenwick can using a modification (Hesling 1956) of the usual process (Goodey 1951), so that there was no loss of new, full cysts. When dry, the cysts were recovered with an aspirator (Hesling 1952).

The cysts obtained from each pot were graded into four size-batches A, B, C, and D, by passing them through a series of sieves of mesh aperture 0.556 mm., 0.401 mm., 0.295 mm., and 0.231 mm. respectively. Very few cysts were lost through the smallest mesh sieve. The number of cysts in each batch from each pot was counted, and the mean egg content of the cysts in each batch was estimated, using, where possible, one hundred cysts. It would be quicker to grade the cysts by sieving and follow this by collecting and counting, but often the cysts are attached to a piece of root which prevents them from reaching the sieve whose mesh will properly retain them. If the cysts are picked out first, those which are still adhering to a root can be separated from it so that they can be sieved correctly.

RESULTS

1. *Effect of Inoculum Levels on Plant Growth*

From the measurements of plant height, it was possible to plot the mean growth curve of the plants at each infestation level (Figs. 1-3), and compare their height at the same time of the year. There is a marked reduction in the plant growth with increased eelworm infestation, and since the growth curves for the oats clearly diverge, the effects of the infestation on this cereal become more apparent as the season progresses.

The susceptibility of the three cereals i.e. the effect of the eelworm on them, appears to be greatest in oats, followed by wheat and barley. A series of curves (Fig. 4) illustrating plant height in July shows this, in that the slope of the curve appears to be greatest for the oats. The effect of the increase of inoculum level on the oats is shown in Fig. 8.

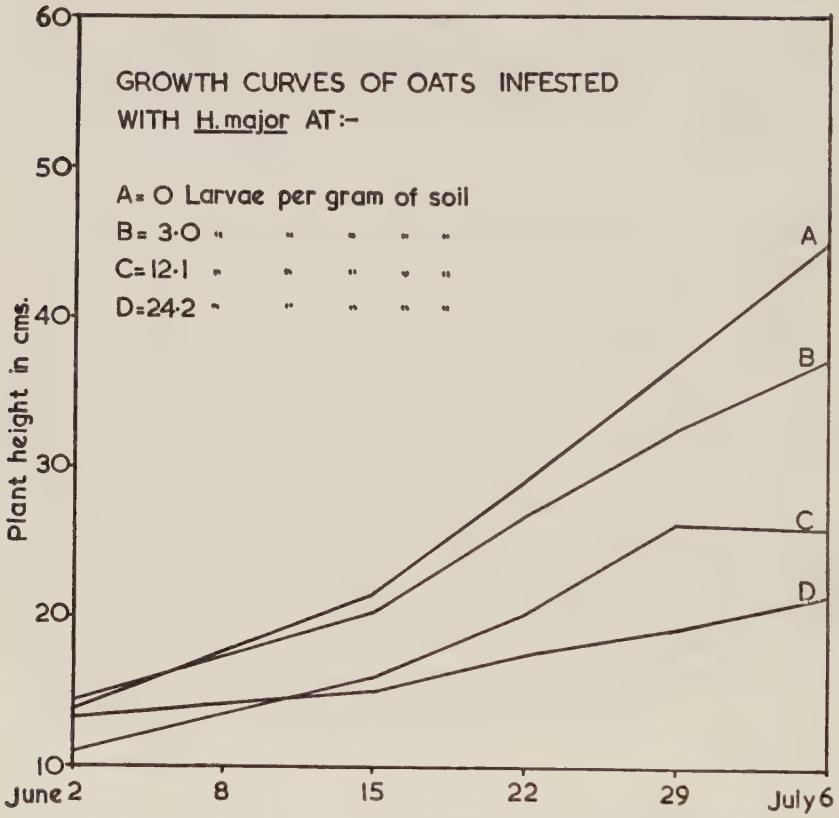

Fig. 1. Effect of different infestations of *H. major* on growth curve of Oats.

2. *The Effect of Inoculum Levels on Final Eelworm Population*

The cyst and egg counts reveal the three items of information listed below. These items are available for each cereal species at each inoculum level.

1. Increase in the eelworm infestation in terms of cyst number and egg number.

2. The percentage of new cysts of each size grade from each pot.
3. The mean egg content of new cysts of similar size.

These three results will now be considered in detail.

Various increase factors for the eelworm infestation may be obtained by the use of the following equations:

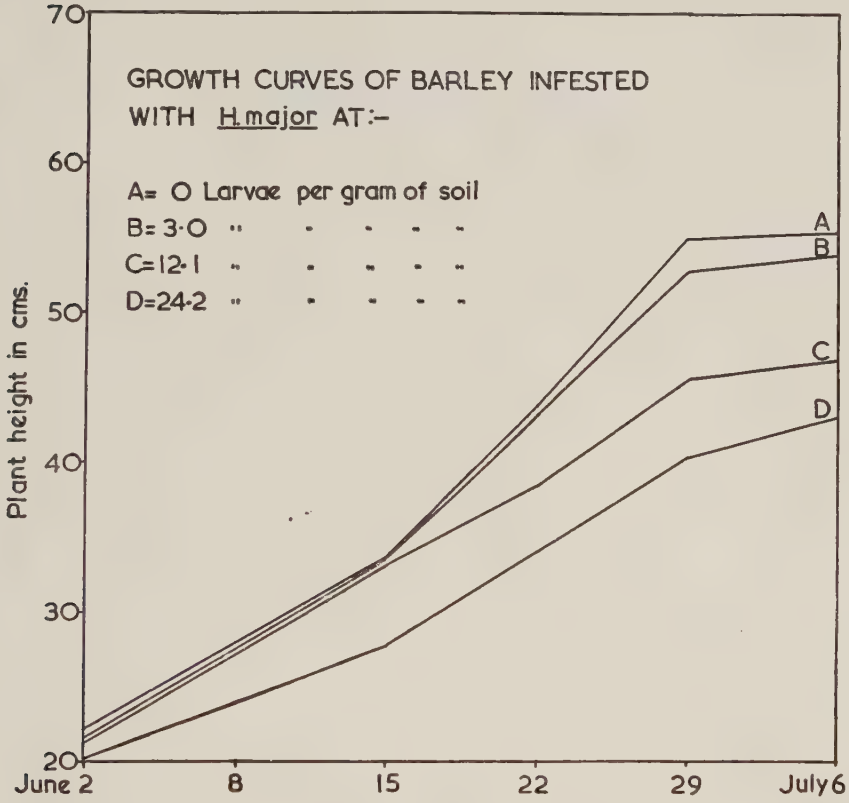

Fig. 2. Effect of different infestations of *H. major* on growth curve of Barley.

a. increase factor $x_1 = \frac{\text{total cysts obtained at end of season}}{\text{original number of cysts}}$

b. " " $x_2 = \frac{\text{number of new cysts produced at end of season}}{\text{original number of cysts}}$

c. " " $x_3 = \frac{\text{total eggs obtained at end of season}}{\text{original number of eggs}}$

d. " " $x_4 = \frac{\text{number of new eggs produced at end of season}}{\text{original number of eggs}}$

Of these four equations, a. and b. may give misleading conclusions, because the denominator in each case is the original number of cysts, and the cyst is not the fundamental unit, c. perhaps gives the best estimate of eelworm increase, but the total eggs at the end of the season may include some of the original eggs, which cannot be said to have increased. In comparing the ability of a plant to cause an increase

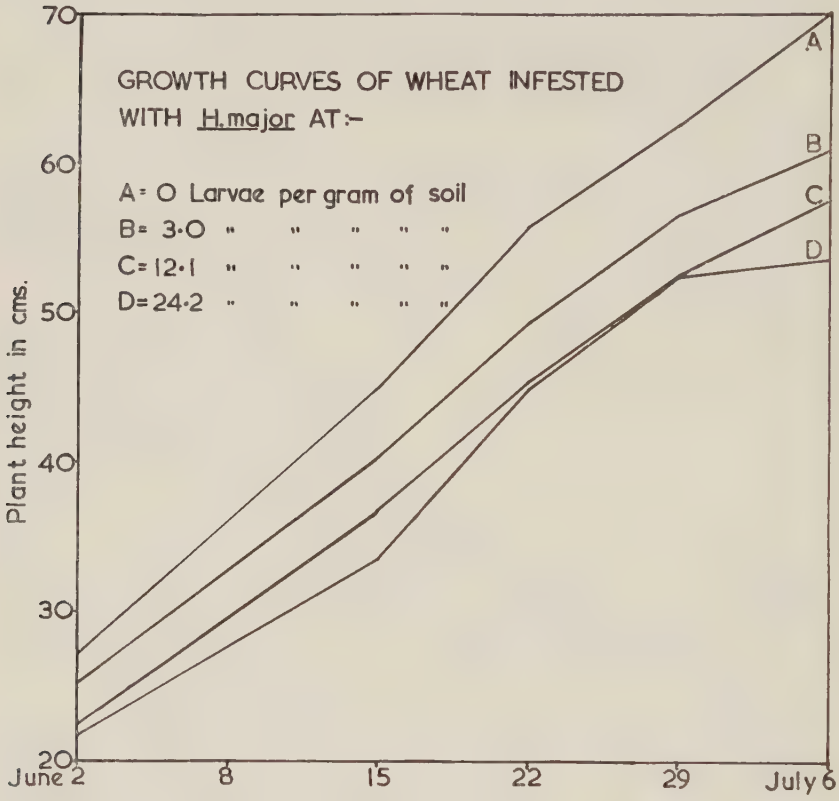

Fig. 3. Effect of different infestations of *H. major* on growth curve of Wheat.

in the eelworm population, d. is the most accurate as it takes into account only the new eggs. Where there has been a high percentage hatch of the original eggs, the values for d. and c. will not differ greatly. Figures 5 and 6 illustrate the mean increase rate at each inoculation level for each cereal, based on b, and d, above. The increase factor based on cyst counts is much higher than that based on egg counts because large full cysts were used to supply the original infestation.

It is often of interest to relate the number of eelworms produced at the end of an experiment to the number which may be expected. Thus, if a 1 : 1 sex ratio is assumed, half the original eggs in a population should produce all the new cysts. The number of new cysts may be expressed as a percentage of half the original number of larvae, and

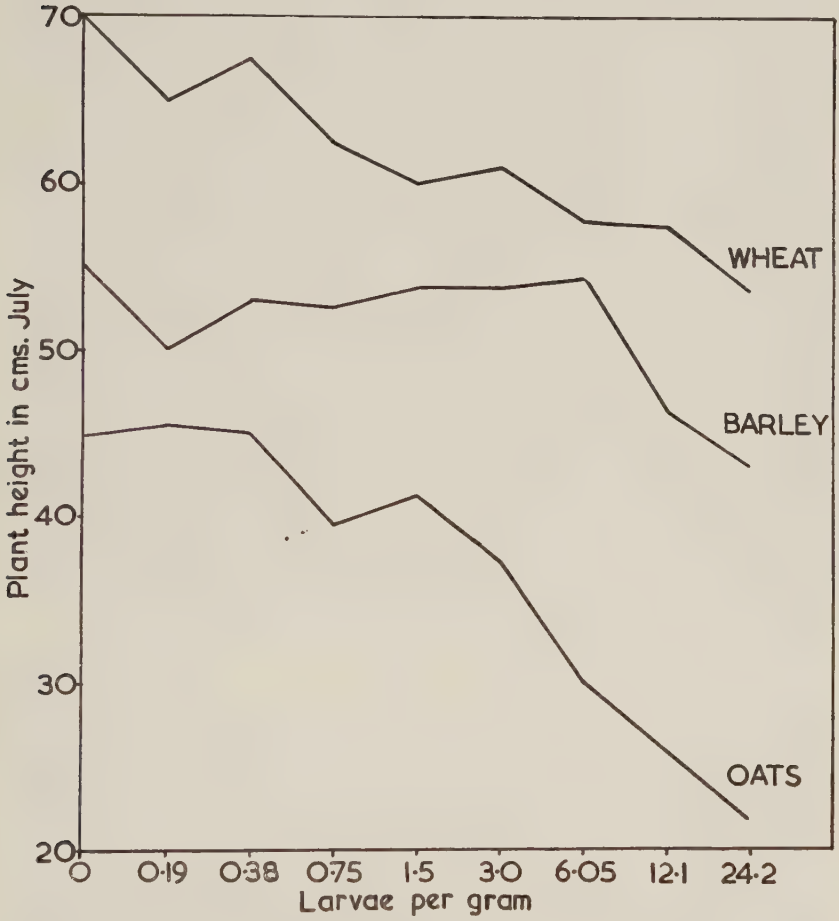

Fig. 4. Graph showing the height of plants of wheat, barley and oats which were grown in different infestations of *Heterodera major*. Each point represents the mean height of 16 plants, the date July 13 1955.

this percentage is termed "cyst-efficiency" by the writer. It may be used to compare the "cyst-efficiency" of different host plants *grown under similar conditions*. Figure 5 shows the "cyst-efficiency" of the cereal plants in this experiment; the "cyst-efficiency" of the plants

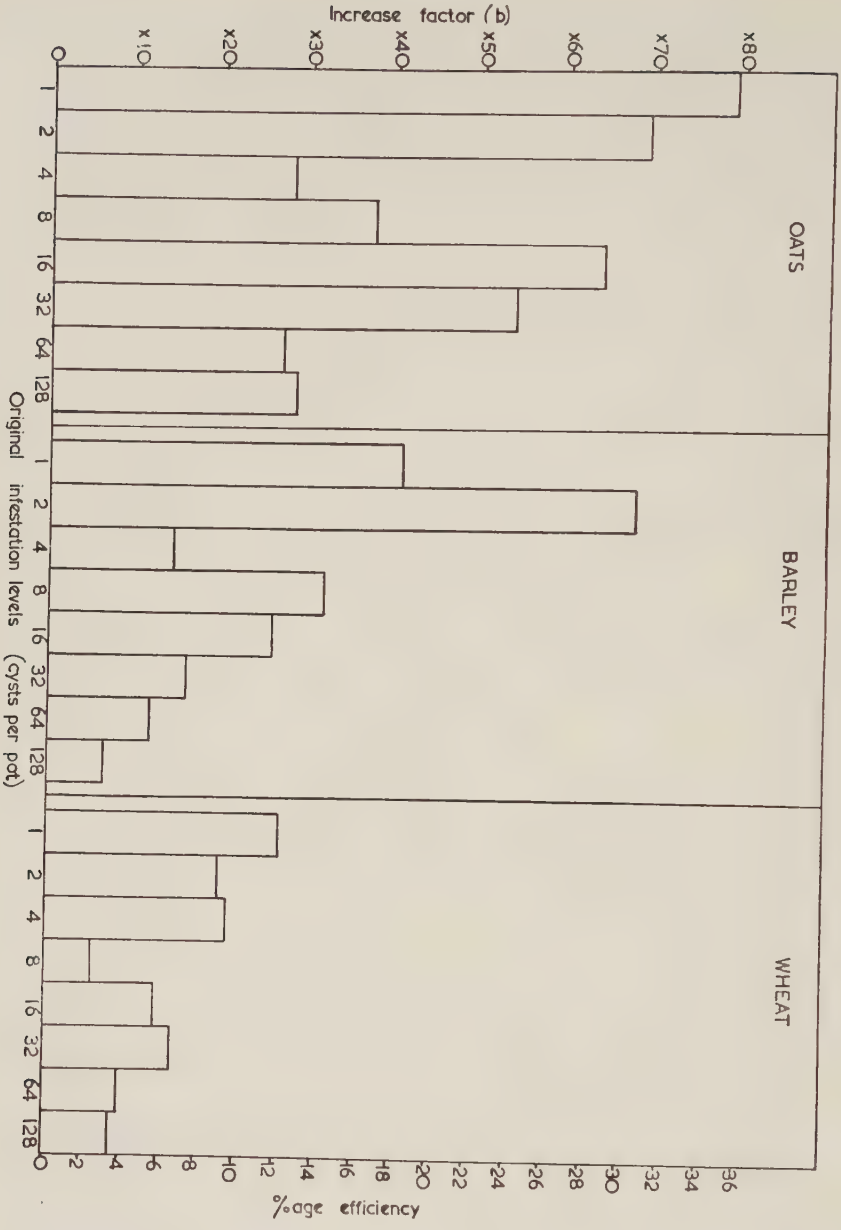

Fig. 5. Histograms (based on cyst counts) showing the effect of different initial populations of *H. major* on eelworm increase and the "cyst-efficiency" (see text) of three cereal hosts.

appears to fall off as the infestation rate increases. The increase factor histograms and "cyst-efficiency" histograms take the same form in this case because it is assumed that the original cysts have similar content

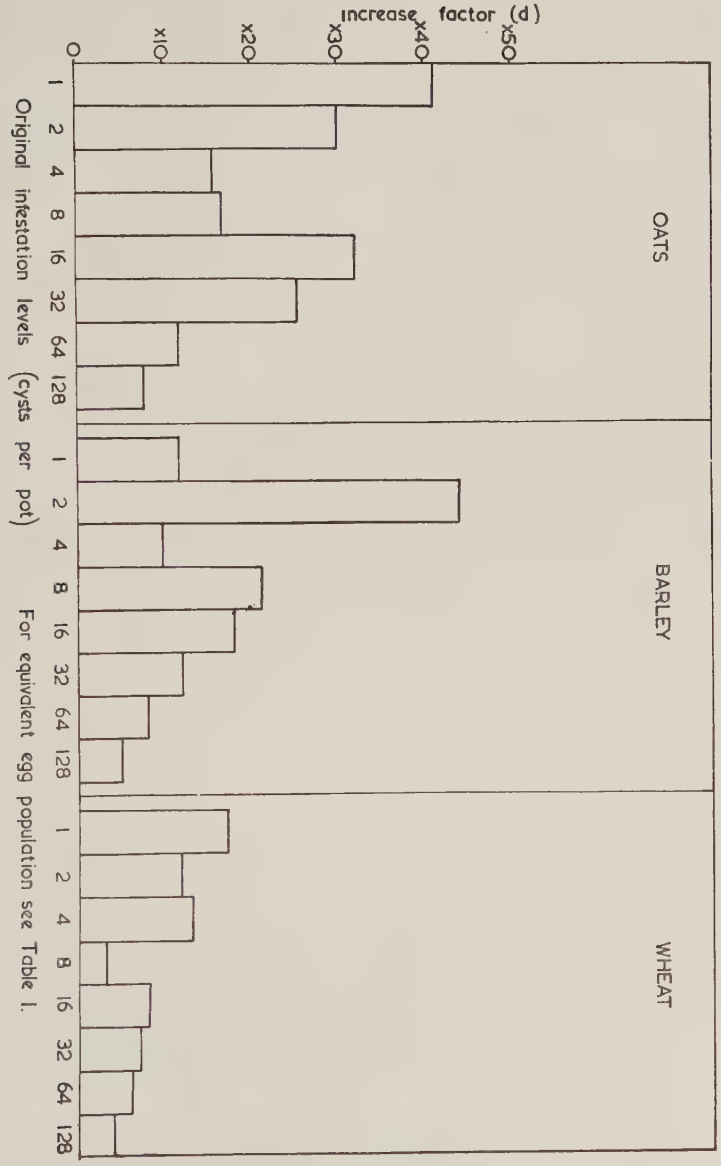

Fig. 6. Histograms (based on egg counts) showing the effect of different initial populations of *H. major* on eelworm increase.

on account of their selection. Nevertheless, the cyst content is variable, and the effect of this may explain the variation in the height of the histograms in Figures 5 and 6, especially at the low infestation levels.

The percentage of new cysts in each size grade differs with inoculum level and with host. This difference is most marked in the largest and smallest cysts; the proportion of large cysts falls, and that of small cysts increases, with increase of inoculum level. Figure 7 illustrates this and shows that barley and wheat produce a higher proportion of large cysts in the final population than do oats.

There is no significant difference, (a) in the egg content of new cysts, between inoculum levels within hosts and within size grades, nor is there any significant difference, (b) in the egg content of new cysts, between hosts within inoculum levels and within size grades.

DISCUSSION

The "cyst-efficiency" observed in this experiment is generally low. The writer has found that the egg content of cysts similar to those used in this experiment falls to less than 40 per cent of its original value between March and late July. Thus, there seems to be a great wastage of larvae which do not become cysts, even at the low inoculum levels. At these levels the intensity of invasion of the roots must be low, not only because of the small number of larvae in the infested soil, but because the invasion of the host plant roots is extended over several months (Schmidt 1931). It seems unlikely that competition between the larvae for nourishment from the roots, or any influence on their sex (Ellenby 1954) play a part in producing the apparent waste of larvae which emerge into the soil.

A comparison of Figures 5 and 6 reveals that the increase factor based on cyst counts is higher than that based on egg counts. This is to be expected in this experiment, for the cysts of the inoculum were large, but the difference in increase factor serves to show that increase factors based on cyst counts vary according to whether the same egg population is from a few large or many small cysts. Even if the initial population is a "field" population, and comprises all sizes of cysts, any increase in the population may produce differences between initial and final population in the percentage of cysts of different size. Thus, if the trend is towards a higher percentage of small new cysts as the initial infestation increases (as is shown in Fig. 7), a series of increase factors based on cyst counts and determined annually during a succession of crops of the same host, must reveal a fall in increase

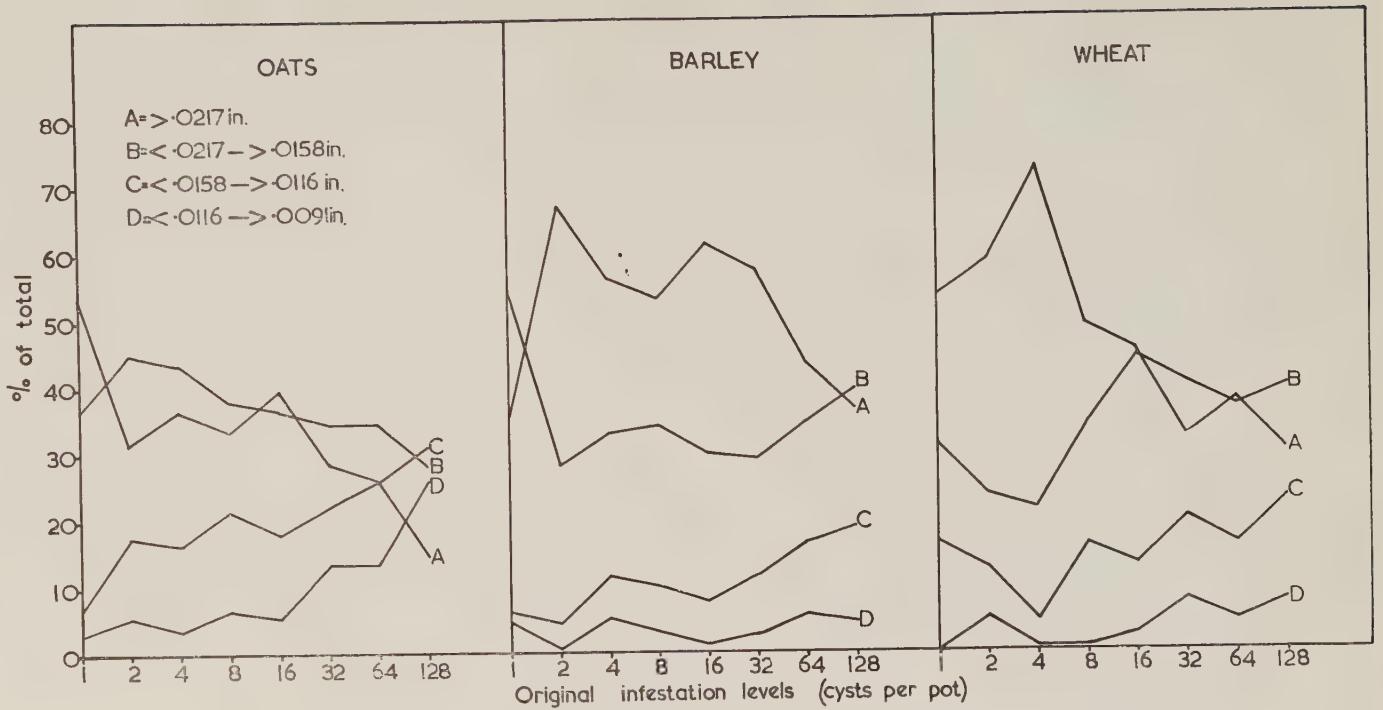

Fig. 7. Graphs showing the number of *H. major* cysts of four different sizes A, B, C and D expressed as a percentage of the total cysts per pot, for three cereal hosts at eight initial infestation levels.

TABLE II
Egg content of New cysts

OATS

Infestation rate cysts/pot		1		2		4		8		16		32		64		128		Mean
Replicate		a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	
Cyst grade	(A	297	334	326	252	376	326	316	282	303	304	324	378	358	334	290	268	310.55
	(B	246	135	186	190	181	248	243	168	246	184	210	184	220	182	211	184	201.05
	(C		111	77	65	131	93	146	90	126	108	109	125	111	110	116	99	108.1
	(D		44	130	35	78	40	42	47	73	51	60	58	57	63	52	45	57.2

BARLEY

Infestation rate cysts/pot		1		2		4		8		16		32		64		128		Mean
Replicate		a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	
Cyst grade	(A		345	328	333	389	279	386	356	366	381	389	366	347	394	308	302	350.75
	(B	238	203	240	212	215	233	213	199	220	226	214	228	185	225	234	222	219.2
	(C		84	147	78	115	83	102	189	107	117	106	90	93	113	124	122	109.65
	(D		35		70	50	54	47	75	80	39	88	44	54	51	63	50	56.4

WHEAT

Infestation rate cysts/pot		1		2		4		8		16		32		64		128		Mean
Replicate		a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	
Cyst grade	(A	397	413	344	328	284	336	345	268	400	396	418	306	314	366	291	324	345.56
	(B	184	219	229	202	197	168	220	150	215	195	202	166	156	214	215	208	196.3
	(C	12	81	132	130	103	177	150	125	129	120	114	82	109	120	118	109	113.1
	(D			75	110	6			100	80	61	52	53	66	52	55	45	61.4

factor different from the true one, which must be based on egg and larval counts.

Table II and Fig. 7 show respectively, that over the range of inocula used, there is no reduction in the egg content of cysts of the same size grade and that the relative number of small new cysts increases with increase of the initial infestation. An estimation of eggs per cyst from a random sample of the total cysts produced from a high and a low infestation level would therefore show a reduction in the mean egg content of the cysts in high as compared with low initial infestations. Thus the trend towards "selflimiting" in a *H. major* population at high infestation levels is associated with the reduction of "cyst-efficiency" of the host plant and with the apparent reduction in the egg content of the total cysts produced. Both these phenomena are probably the result of one or more unknown factors. The difference in the percentage of cysts of given size produced from various infestation levels and by different hosts may affect the use of a sieving technique (Winslow 1955) for extracting most of the "hatchable" larvae from field populations.

Attempts to determine resistance of cereals (i.e. their ability to increase the eelworm population) to *H. major* by final cyst and egg counts may give misleading results unless the initial infestation levels to which the plants are exposed are similar. Such a test of resistance does not directly measure the susceptibility of the host plant (i.e. the effect of the eelworm on it). If differences in resistance to *H. major* between cereal varieties are slight, any technique for varietal testing must be sensitive. Such a technique (which would facilitate comparisons between variety trials at different places) should be suitably replicated, involve a series of infestation levels (including control) and have a suitable variety as a standard. An experiment of this type would probably have to be carried out in pots of soil to achieve control of such factors as soil type, fertility and moisture. Ideally, the possibility of biotypes of *H. major* necessitates the use of a standard eelworm population.

For cereals, plant height measurements may be used to estimate the susceptibility of the host plant to injury by *H. major*. Other symptoms, such as discolouration of the leaves, weak growth, delayed or early flowering are difficult criteria to use, because they are not quantitative. Intervarietal difference in plant growth and habit make essential the use of a zero infestation control.

It is noticeable from figures 4 and 8 that the population of

H. major which can cause damage to oats is relatively low compared with the size of damage-producing populations of *H. rostochiensis* on potato (CARROLL and MCMAHON 1935). In this experiment, 24 larvae per gram of soil affected the oat plants so severely that they would have represented crop failure to a farmer. It is very probable, however, that such an effect was accentuated by concentrating plant roots and the eelworms in pots, and it would be unwise to extrapolate from the results of this experiment about what might happen in the field, where different conditions apply.

ACKNOWLEDGMENTS

The writer wishes to thank Mr. J. H. A. Dunwoody for help with statistics, Miss J. Daniels for help with soil samples, and his colleagues at Rothamsted for advice during the course of the work.

ZUSAMMENFASSUNG

Hafer, Weizen und Gerste wurden getrennt in Töpfen angezogen, die in verschiedenen Masse mit Hafernematoden, *Heterodera major* O. Schmidt 1930, infiziert waren. Die Wirkung auf die Wuchshöhe der Pflanze nahm mit steigender Infektion zu; am empfindlichsten war der Hafer, es folgten Weizen und Gerste. Geringe Verseuchungen führten zu einer stärkeren Vermehrung der Nematoden und umgekehrt. Bei Gerste entwickelte sich ein relativ grösserer Anteil an grossen Cysten. Die neuen Cysten enthielten zwischen 6 und 413 Eier; weder die Getreideart noch die Stärke der ursprünglichen Verseuchung hatten irgendeinen Einfluss auf den Inhalt von Cysten derselben Grösse. Da jedoch in den Töpfen mit starker Anfangsinfektion eine geringere Anzahl von grossen Cysten entstanden, fiel der durchschnittliche Gehalt der neuen Cysten mit steigender Anfangsinfektion ab.

Die Ergebnisse dieser Versuchsreihe verweisen auf eine günstige Methode, um die Widerstandsfähigkeit und Empfindlichkeit der Getreidesorten gegen *H. major* genau zu prüfen.

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Fig. 8. Effect of a series of different initial infestations of *H. major* on oats. Inoculum level increases from right to left: 0, 1, 2, 4, 8, 16, 32, 64, 128 cysts per pot.

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ERRATUM

CEREAL ROOT EELWORM

My attention has been drawn to a recommendation made by the International Commission on Zoological Nomenclature at its 9th meeting in Paris in July 1948 and quoted in the Bulletin of Zoological Nomenclature **4** (10/12), 254 (1950). This is "that the name of an author is not to be placed in brackets (parentheses) when a trivial name originally published as the name of a species is later treated as the name of a subspecies and *vice versa,* so long as that species or subspecies is cited in its original nominal genus." The correct citation of the name of the cereal root eelworm is therefore "*Heterodera major* O. Schmidt, 1930" and not "*Heterodera major* (O. Schmidt, 1930) Franklin, 1940" as stated in my note on this subject on p. 151 of Nematologica II (2).

M. T. FRANKLIN

Nematologica II (1957): 300-305. — Leiden, E. J. Brill

DIE WIRKUNG IONISIERENDER STRAHLEN AUF DIE ENTWICKLUNG VON *HETERODERA ROSTOCHIENSIS* UND *H. SCHACHTII* [1])

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Die derzeitigen Kenntnisse über den Einfluss verschiedener Strahlen auf Nematoden sind sehr begrenzt. Nur vereinzelt sind Bestrahlungsversuche durchgeführt worden; so stellte GOFFART (1953) fest, dass Nematodenzysten eine hohe Dosis Roentgenstrahlen vertragen. FASSULIOTIS und SPARROW (1955) machten ebenfalls Versuche mit Roentgenstrahlen, um an Kartoffelknollen haftende Zysten von *Heterodera rostochiensis* abzutöten. Auch sie bemerkten eine grosse Widerstandsfähigkeit der Nematoden gegenüber der Strahlung. Versuche von FULDNER (1955) mit ultraviolettem Licht gehören nur bedingt in diesen Zusammenhang, da diese Strahlen einen anderen biologischen Wirkungsmechanismus haben. Bestrahlungsversuche mit radioaktiven Substanzen sind an Nematoden bisher noch nicht durchgeführt worden.

In Fortsetzung der Untersuchungen von GOFFART (1953) wurden Zysten von Kartoffelnematoden (*Heterodera rostochiensis* Wollenw.) und Rübennematoden (*Heterodera schachtii* Schmidt) den Strahlen eines Radiumpräparates mit 50 mg reinem Radium ausgesetzt und anschliessend die Vitalität der bestrahlten Tiere in Schlüpf- und Infektionsversuchen geprüft und die Entwicklung über 2 Generationen beobachtet. Bei der verwendeten Versuchsanordnung betrug die abgegebene Strahlendosis etwa 400 r (Roentgeneinheiten) pro Stunde. Das Spektrum der Gammastrahlen besteht im Wesentlichen aus Strahlen von 88 KeV (Kilo-Elektronenvolt) und 12 KeV und entspricht einer Ionisierungsdichte von 200-300 Ionen pro μ. Ferner gibt das Präparat noch schnelle Neutronen von mehreren MeV (Million-Elektronenvolt) ab. Die biologische Wirksamkeit ionisierender Strahlen beruht, wie der Name sagt, primär auf der Freimachung von Elektronen aus elektrisch neutralen Atomen und Molekülen. Ausserdem

1) Eingegangen: 12. Mai 1957.

sind sie in der Lage, den Energiezustand der Atome und Moleküle zu verändern, sie „anzuregen". Die ionisierten oder aktivierten Teilchen sind dann zu besonderen Reaktionen befähigt.

Da keine Erfahrungen über die Wirkung derartiger Strahlen auf Nematoden vorlagen, wurde zunächst entsprechend der Technik bei der Arbeit mit Roentgenstrahlen nur kurzzeitig, d.h. nach Minuten bestrahlt. Im anschliessenden Schlüpfversuch konnte jedoch keinerlei Wirkung festgestellt werden. Auch bei einer Bestrahlungsdauer von einigen Stunden zeigten sich keine Unterschiede. Erst bei 72 Stunden machte sich ein deutlicher Einfluss bemerkbar. Die Behandlung wurde dann weiter ausgedehnt und maximal eine Bestrahlungsdauer von 196h angewandt.

Nach der Bestrahlung wurden die Zysten makroskopisch und mikroskopisch untersucht. In keinem Fall konnten äusserlich sichtbare Veränderungen wahrgenommen werden. Die Zysten waren durch die Bestrahlung auch nicht selbst radioaktiv geworden, wie mit dem Geiger-Müller-Zählrohr nachgewiesen wurde. Die im Folgenden beschriebenen Erscheinungen sind also unmittelbar durch die äusserliche Bestrahlung hervorgerufen und nicht etwa Folgen einer induzierten eigenen Strahlungsaktivität.

Es erfolgte dann die Untersuchung des Zysteninhaltes. Alle Zysten stammten aus einheitlichen Populationen und waren so ausgesucht, dass sie zum Zeitpunkt der Behandlung fast ausschliesslich schlüpffähige Larven enthielten. Auch an den Larven waren keine sichtbaren Schädigungen wahrnehmbar. Für die Schlüpfversuche wurden Gruppen von jeweils 10 Zysten der beiden Arten und entsprechende Kontrollen in 2 cm Ablaufwasser im Thermostaten bei 25° C angesetzt. Die geschlüpften Larven wurden über 30 Tage hinweg ausgezählt. Die Ergebnisse dieser Zählungen sind in Tabelle I zusammengefasst.

Tabelle I

Durchschnittliche Anzahl der innerhalb von 30 Tagen aus jeweils 10 Zysten geschlüpften Larven. Strahlendosis etwa 400 r pro Stunde.

Bestrahlungsdauer	72h	96h	120h	144h	168h	196h
H. rostochiensis						
bestrahlt	1083	1432	1410	1219	1750	1134
Kontrolle	832	720	1012	1222	1500	1016
H. schachtii						
bestrahlt	616	1127	1301	1583	711	710
Kontrolle	2080	1774	2252	3446	2860	1379

Zunächst ist bemerkenswert, dass auch die höchste Strahlendosis, die insgesamt etwa 78400 r betrug, noch nicht 100 %ig tödlich war. Höher organisierte Tiergruppen zeigen bei derartigen Energiemengen schon schwerste Schädigungen. So treten z.B. bei weissen Mäusen nach einer einmaligen Verabreichung von 100-150 r die ersten akuten Todesfälle auf. Bei 1000-2000 r sterben fast alle behandelten Tiere nach 3-4 Tagen ab (Rajewski 1954). Über die Wirkung von Langzeitbestrahlungen, wie sie den vorliegenden Versuchen zugrunde liegen, ist bei Wirbellosen noch sehr wenig bekannt. Bei Säugetieren ruft bereits eine Dosis von wenigen Roentgeneinheiten je Tag eine merkliche Verkürzung der Lebenszeit hervor (Blair 1954, Rajewski 1954). Natürlich sind diese an Warmblütern gewonnenen Daten nur begrenzt auf andere Tiergruppen anwendbar. Trotzdem kann man sagen, dass die Nematoden gegen ionisierende Strahlen sehr widerstandsfähig sind.

Wenn in den Versuchen auch keine vollständige Abtötung erreicht wurde, so zeigt sich in den Ergebnissen der Schlüpfversuche doch eine deutliche Wirkung der Bestrahlung auf die Nematoden. Sie war bei den beiden geprüften Arten unterschiedlich. Die Larven des Kartoffelnematoden werden zu einem stärkeren Schlüpfen angeregt, die Larven des Rübennematoden demgegenüber stark gehemmt. Das wird besonders deutlich, wenn man die aus den bestrahlten Zysten geschlüpften Larven in Beziehung setzt zur Anzahl der Larven in den Kontrollen (Tabelle II).

Tabelle II

Anteil der Larven aus bestrahlten Zysten in Prozent der Larven aus den Kontrollzysten

Bestrahlungsdauer	72h	96h	120h	144h	168h	196h
H. rostochiensis bestrahlt in % der Kontrollen	130%	198%	139%	99%	116%	111%
H. schachtii bestrahlt in % der Kontrollen	29%	63%	57%	45%	24%	51%

Eine sehr starke Schlüpfhemmung wurde in einem Nebenversuch auch bei Wurzelgallenälchen (*Meloidogyne incognita*) beobachtet. Schon bei einer Bestrahlungszeit von 48h betrug der Anteil der Larven aus den bestrahlten Eimassen nur 26% der Gesamtzahl. Sie schei-

nen also noch empfindlicher zu sein als *Heterodera schachtii*. Über den Mechanismus der Hemmung oder der Förderung ist noch nichts bekannt. Es handelt sich nicht um eine zeitliche Verschiebung des Schlüpfens, sondern um eine allgemeine Hebung bzw. Senkung der Schlüpfrate. In Abb. 1 sind als Beispiel für den zeitlichen Verlauf des Schlüpfens die täglichen Schlüpfraten nach einer 72stündigen Bestrahlung der Zysten graphisch dargestellt. Die Abbildungen zeigen einen völlig gleichartigen Verlauf des Schlüpfens bei behandelten und unbehandelten Zysten. Die Anzahl der Larven ist nur entsprechend gestiegen bzw. gesunken.

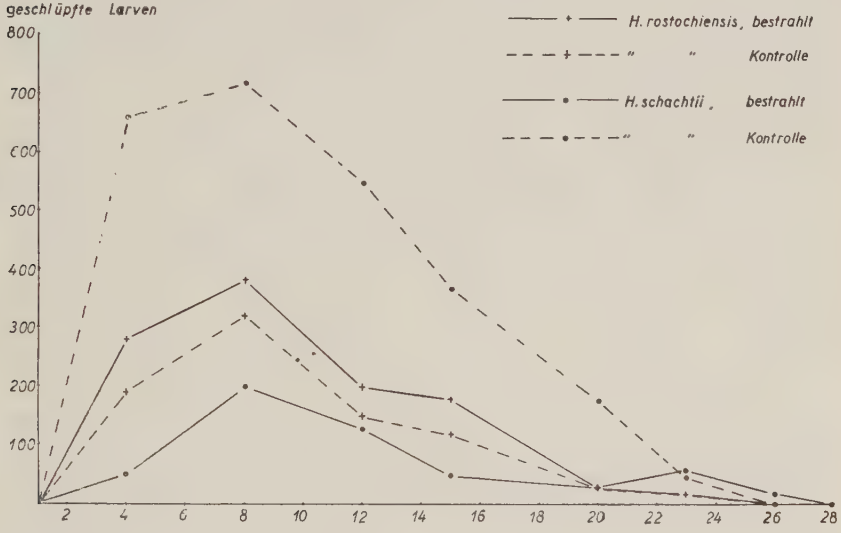

Abbildung 1. Schlüpfraten der Larven von *Heterodera rostochiensis* und *H. schachtii* nach 72^{h} Bestrahlung.

Zur weiteren Prüfung der Vitalität und Virulenz der behandelten Tiere wurden Infektionsversuche angesetzt. Töpfe mit Kartoffeln oder Rüben in sterilisierter Erde wurden mit je 25 Zysten infiziert. In bestimmten Zeitabständen erfolgte die Untersuchung von Wurzeln auf eingewanderte Larven nach Färbung mit Lactophenol-Säurefuchsin. Dabei konnte festgestellt werden, dass sich die Bestrahlung auf die Infektionsfähigkeit beider Nematodenarten negativ ausgewirkt hatte. Zunächst war das Eindringen der Larven, die aus den behandelten Zysten stammten, in die Wurzeln merklich verzögert. Die ersten Larven waren hier immer einige Zeit später zu finden als in den Kon-

trollen. Ausserdem war auch die Anzahl der eingedrungenen Larven wesentlich geringer. Das liess sich bei den Rübennematoden erwarten. Aber auch bei den Kartoffelnematoden traten trotz der Schlüpfförderung die gleichen Erscheinungen auf. Daraus ist zu schliessen, dass es sich bei der Erhöhung der Schlüpfrate durch Bestrahlung nicht um eine allgemeine Steigerung der Vitalität und Virulenz handelt, sondern eher um eine krankhafte Reizung. Der Verzögerung der Infektion bei den Larven aus bestrahlten Zysten entsprach eine weitere Verlangsamung im Laufe der Entwicklung. Dadurch wurde die zeitliche Differenz im Auftreten der einzelnen Phasen zwischen den behandelten und den unbehandelten Tieren immer deutlicher. Als Beispiel sei die Färbung als Merkmal der Zystenreife von Kartoffelnematoden bei der Auswertung eines Topfes mit 96h lang bestrahlten Zysten wiedergegeben (Tabelle III).

Tabelle III

Zystenreife von H. rostochiensis bei Auswertung eines Topfes; Elternzysten 96h lang bestrahlt

Zystenfarbe	weiss	gelb	bräunlich-braun	Gesamtzahl d. Zysten
bestrahlt	27 %	38 %	40 %	94
Kontrolle	0,4 %	10 %	89 %	418

Aus der Tabelle geht hervor, dass in den Kontrollen der weitaus grösste Teil der Zysten mit Erreichen einer bräunlichen Färbung die Entwicklung abgeschlossen hatte, während ein grosser Prozentsatz der Nachkommen der bestrahlten Zysten das Reifestadium noch nicht erreicht hat. Neben der Entwicklungsverzögerung zeigt die Tabelle auch die geringe Vermehrungsrate der behandelten Tiere. Sie war nicht immer so gering wie in diesem Versuch, betrug aber im Durchschnitt nur $^1/_3$ der normalen Vermehrung in den Kontrollen. Eine Verschiebung des Geschlechtsverhältnisses als Folge der Bestrahlung wurde nicht beobachtet.

Die starke Entwicklungshemmung liess es möglich erscheinen, dass die Larven aus den bestrahlten Zysten gar keine normalen Zysten mit infektionsfähigen Inhalt gebildet haben. Diese Vermutung wurde aber durch die Untersuchung des Zysteninhaltes nicht bestätigt. Zwischen den neugebildeten Zysten in den Kontrollen und in Töpfen mit behandelten Tieren bestanden keine sichtbaren Unterschiede. Indirekt machte sich allerdings doch eine gewisse Vitalitätsminderung bei den Nachkommen der bestrahlten Zysten bemerkbar. Sie verpilzten näm-

lich im Thermostaten sehr viel schneller und stärker als die Kontrollen. Zur weiteren Prüfung wurden mit den neugebildeten Zysten ebenfalls Infektionsversuche angesetzt. Dabei konnten grundsätzlich die gleichen Beobachtungen gemacht werden wie bei der Elterngeneration. Infektion und Entwicklung waren verzögert und der Vermehrungsfaktor noch geringer.

Über das Zustandekommen der verminderten Vermehrungsfähigkeit können vorerst nur Vermutungen angestellt werden. Bei der stark mutationsauslösenden Wirkung ionisierender Strahlen liegt der Gedanke nahe, dass durch die Bestrahlung die Mutationsrate stark gesteigert wurde. Da die Nematoden zu den sogenannten zellkonstanten Tieren gehören und ein sehr geringes Regulations- und Regenerationsvermögen besitzen, könnten hier Mutationen häufiger letal sein als bei anderen Organismen. Dadurch würde eine geringere Vermehrung bedingt werden. Der Nachweis genetischer Veränderungen ist bei Nematoden z.Zt. noch sehr schwierig, da die Kenntnisse der erbmässigen Verankerung einzelner Merkmale äusserst gering sind. Versuchsweise wurde die Grösse der Zysten gemessen und mit den Kontrollen verglichen. Die gefundenen Werte ergaben aber keine gesicherten Unterschiede. Offenbar ist die Grösse kein sehr geeignetes Merkmal, da sie bei Nematoden ohnehin stark schwankt.

Die Versuche werden fortgesetzt.

SUMMARY

Cysts of the potato root eelworm and the beet eelworm were irradiated by radium for different times up to 196 hours. The radiation dose rate was 400 r/h (r = Roentgen). The spectrum mainly consisted of gamma-rays of 12 and 88 KeV and of neutrons of some MeV. Compared with data for other animals in the literature, the nematodes showed a remarkable resistance to the rays. Treatments of less than 72 hours had no apparent effect. After longer exposure, the hatching of larvae from the cysts of the beet eelworm was reduced whereas in the potato root eelworm many more larvae hatched from irradiated cysts than from untreated ones. In spite of this the development of larvae in both species was retarded and the rate of reproduction reduced. Mutative variations of body size as a consequence of the radiation were not observed.

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APHELENCHOIDES COMPOSTICOLA N.SP. AND *A. SAPROPHILUS* N.SP. FROM MUSHROOM COMPOST AND ROTTING PLANT TISSUES [1])

BY

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The genus *Aphelenchoides* includes not only important parasites of higher plants but also species inhabiting soil and decaying plant tissues. The most commonly recorded species from soil and plant remains is *A. parietinus* (Bastian, 1865) Steiner, 1932. For some time it has been the opinion of nematologists that this probably comprises a group of closely related species.

The first step towards elucidating the position was the re-description of *A. parietinus* obtained from the same place as Bastian's specimens (Franklin, 1955). The second is to describe two new species closely resembling *A. parietinus,* the former being commonly found in the compost prepared from straw and dung, which is used in the cultivation of mushrooms, and the latter in rotting plant tissues and soil.

Measurements given in the following descriptions were all made on specimens relaxed in water by gentle heat and transferred to TAF fixative, except for measurements of stylets and spicules, when some specimens were in fixative and some in glycerine.

Aphelenchoides composticola n.sp.
(Fig. 1, A-D)

This species is one of the causes of failure of mushroom beds (Moreton, John & Goodey, 1956). Males and females have been found in compost from a number of places where mushrooms had failed to grow. Populations from several sources have been cultured on agar plates with mushroom mycelium (*Agaricus hortensis* Cooke) and with *Alternaria tenuis* Nees. This is therefore a fungivorous species.

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1) Received for publication: May 12, 1957.

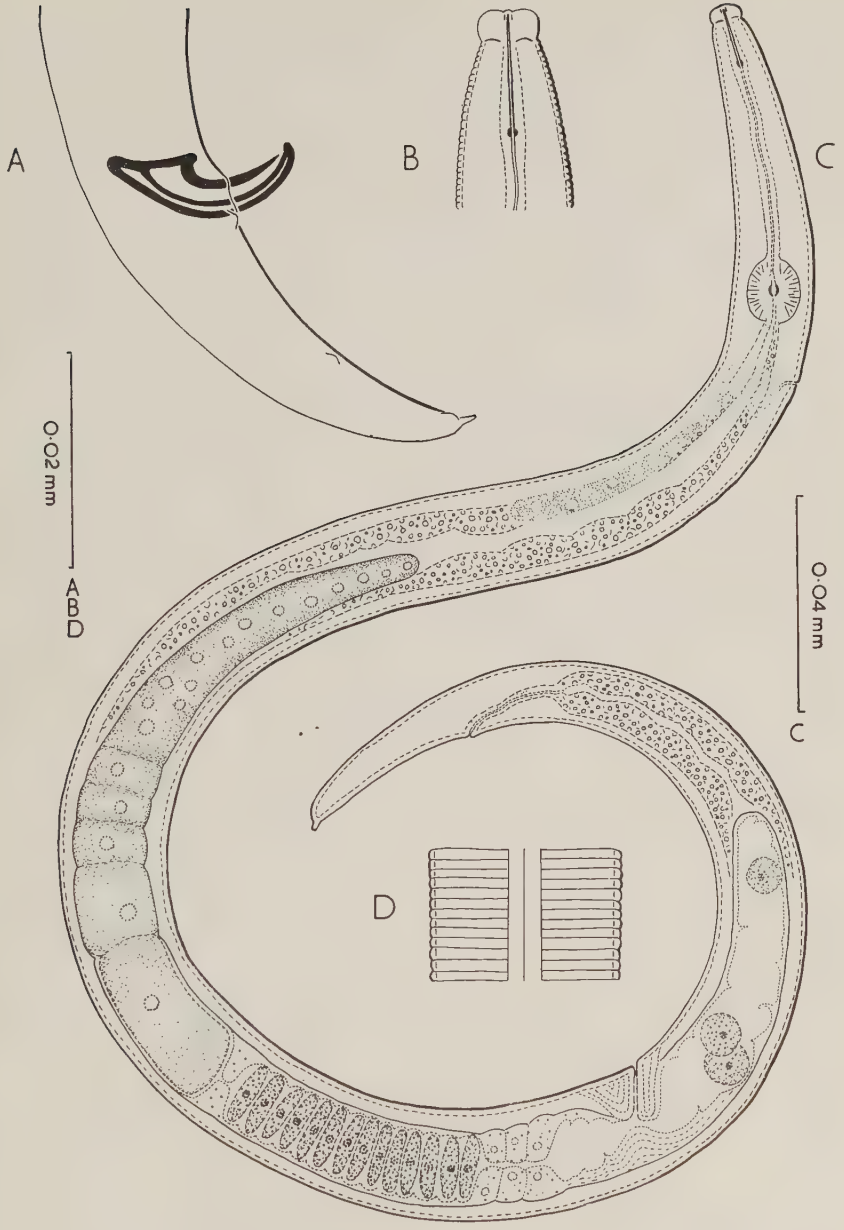

Fig. 1. *Aphelenchoides composticola* n.sp. A. Male tail. B. Head. C. Female. D. Annules and lateral field.

Female Length: 517 μ (447-614 μ) (n = 24)
Breadth: 15 μ (11-19 μ) (n = 24)
a — 34 (30-42) (n = 24)
b — 9 (8-10) (n = 21)
c — 14 (11-17) (n = 23)
V — 70% (67-72%) (n = 24)
Stylet: 11 μ (n = 2)

The body is slender with offset lips. The head is without annules or papillae. The body annules are 0.9 μ- 1.0 μ wide and the lateral field occupies one fifth of the body width and has three longitudinal incisures. These are reduced to two, then one, posteriorly on the tail and anteriorly in the region of the oesophageal bulb. The tail tapers to a rounded point bearing a terminal mucro which is usually ventral (Fig. 1 C). The vulva is a transverse slit and is not prominent. The anus is more easily seen; the anterior lip is slightly prominent. The stylet is made up of anterior conical and posterior cylindrical parts with three small basal swellings. There are short guiding rods anteriorly. The stylet lumen is continuous with the oesophageal lumen which leads back to the well-developed muscular median bulb. The points of entry of the oesophageal gland ducts are visible as interruptions of the lumen of the bulb in front of and behind the median valve. Behind the bulb the oesophagus widens into the intestine. The oesophageal glands form a lobe overlying the intestine dorsally, about three body-widths long. The nerve ring surrounds the beginning of the intestine and the excretory pore is situated ventrally at about the same level or a little behind. The ovary is a single row of cells for most of its length, without flexures, starting at a point one or two body-widths behind the end of the oesophageal glands. Behind the ovary the spermatheca is often found tightly packed with sperms compressed into a single row of disc-like bodies. The post-vulval sac also serves as spermatheca, reaching one half to two thirds the distance to the anus.

Eggs Length: 46 μ. Breadth: 19 μ. Ratio L/B: 2.4 (n = 9)

Male Length: 489 μ (412-581 μ) (n = 25)
Breadth: 15 μ (11-18 μ) (n = 25)
a — 34 (28-41) (n = 25)
b — 8.5 (7-9) (n = 24)
c — 15 (11-20) (n = 25)
Stylet: 11 μ (n = 3)
Spicules: dorsal limb 21 μ (n = 5)

Males are easily distinguished from females when dead by the hooked tail and slightly smaller size. The testis is a single row of cells stretching forward nearly to the end of the oesophageal glands. The paired spicules are typically aphelenchoid: the dorsal limb is smoothly curved and the tip of the ventral limb reaches nearly to that of the dorsal. The transverse bar joining the limbs proximally has a ventral protuberance. There is no gubernaculum but the usual three pairs of caudal papillae are present, one adanally, one midway along the tail and the third at the base of the mucro.

Relationships

A. composticola differs from *A. parietinus* in size and proportions, in the length of the stylet (11.75 μ in *A. parietinus*), the number of incisures on the lateral line (four in *A. parietinus*) and the occurrence of males.

Type habitat: Mushroom compost.

Type locality: Ormskirk, Lancashire.

Type material: Collection no. 86/15/TC and slides nos 86/15/1-6, Nematology Department, Rothamsted Experimental Station.

Aphelenchoides saprophilus n.sp.
(Fig. 2, A-D)

This nematode is very frequently found, amongst others, in rotting plant tissues which have been in contact with soil, such as roots, stems, tubers and bulbs. It is very similar to *A. parietinus* and has probably been referred to that species many times in the past. Like *A. composticola* it is easily cultured on agar plates with *Alternaria tenuis*, and males are common.

Female

Length:	546 μ	(454-623 μ)	(n = 20)
Breadth:	19.5 μ	(16.5-28 μ)	(n = 20)
a —	28	(26-33)	(n = 20)
b —	9.5	(8-12)	(n = 19)
c —	16	(12-18)	(n = 17)
V	69%	(66-70%)	(n = 20)

Stylet: 11μ (n = 5)

This species strongly resembles *A. composticola* but is somewhat stouter in proportion. The body annules are about 0.7-0.9 μ. The lateral field is one fifth of the body width wide, with four longitudinal incisures. The tail is slightly more tapering than that of *A. com-*

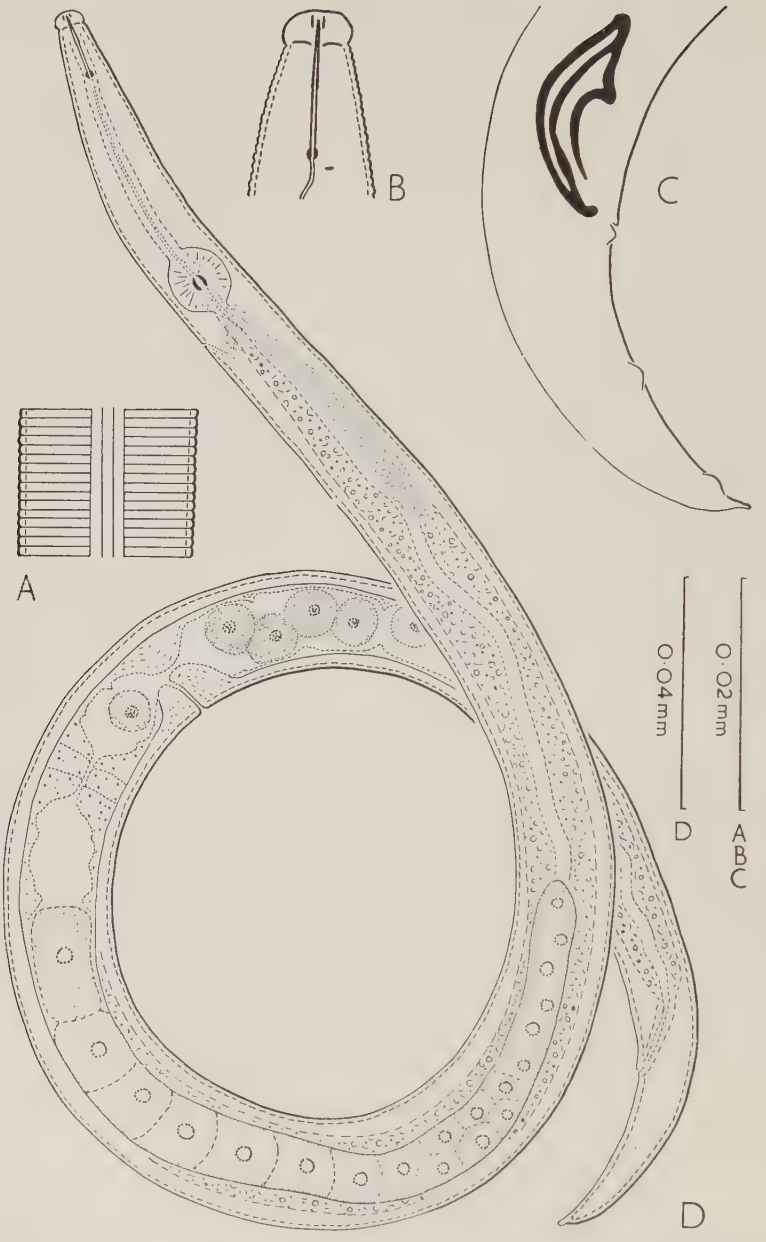

Fig. 2. *Aphelenchoides saprophilus* n.sp. A. Annules and lateral field. B. Head. C. Male tail. D. Female.

posticola. The alimentary tract and reproductive organs are essentially similar to that species.

Eggs Length: 46 μ Breadth: 18 μ Ratio L/B: 2.6 (n = 7)

Male Length: 545 μ (476-627 μ) (n = 20 for all values except stylet and spicules)

Breadth: 18 μ (16-21 μ)
a — 30 (27-34)
b — 9 (8-11)
c — 15 (13-18)

Stylet: 12 μ (n = 4)

Spicules: dorsal limb 23 μ (n = 4)

The male of this species is distinguished from that of *A. composticola* by having four incisures on the lateral line instead of three, and by the shape and slightly larger size of the spicules. The latter are aphelenchoid in form but the dorsal limb is stouter and less smoothly curved and its tip is slightly knobbed. The ventral limb is shorter in proportion to the dorsal and the protuberance at the dorsal end of the transverse bar joining the limbs is more marked (compare Fig. 1 A with 2 C). The tail is similar to that of *A. composticola* with the usual three pairs of papillae and a well-developed mucro.

Relationships

A. saprophilus has spicules very similar in shape to those of *A. blastophthorus* but distinctly smaller (dorsal limb 23 μ as compared with 28 μ). The mouth stylet also is shorter (12 μ as compared with 17.5 μ), and the body broader in proportion to its length (a = 30 as compared with 41). From *A. parietinus* it differs in being slightly longer and more slender and in having a relatively shorter egg (L/B = 2.6 as compared with nearly 4), and finer annulations (0.7-0.9 μ as compared with 1.0-1.3 μ). It can easily be cultured on an agar-fungus medium while *A. parietinus* is hard to establish. Males of *A. parietinus* have never been found either in its natural habitat or in the one culture which the author has been able to establish.

Type habitat: Soil.

Type locality: Docking, Norfolk.

Type material: Collection no 86/16/TC and slides nos 86/16/1-6, Nematology Department, Rothamsted Experimental Station.

As mentioned above, both *A. composticola* and *A. saprophilus* are easily cultured on fungus-agar plates. It has been found that after

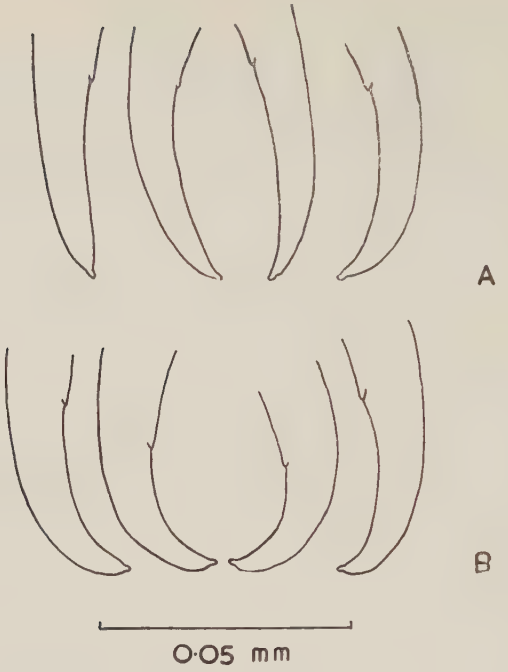

Fig. 3. A. Tails of female *A. saprophilus.* n.sp. B. Tails of female *A. parietinus.* (Bastian, 1865) Steiner, 1932.

culture for some weeks the mean size of the population may have altered considerably. Some examples are given in Table I.

TABLE I

Measurements of Aphelenchoides spp. after various periods of cultivation on agar-fungus medium

	A. composticola			*A. saprophilus*		
	Original population	11 weeks culture	9½ months culture	Original population	6 weeks culture	9 months culture
Female						
Mean length	517	528	592	546	494	421
Range μ	(447-614)	(462-623)	(512-648)	(454-623)	(412-581)	(370-481)
Mean *a*	34	31	32	28	27	25
Range	(30-42)	(28-35)	(28-38)	(26-33)	(22-31)	(20-29)
Male						
Mean length	489	513	578	545	507	423
Range μ	(412-581)	(423-650)	(494-679)	(476-627)	(423-562)	(370-475)
Mean *a*	34	32	32	30	30	25
Range	(28-41)	(26-38)	(26-41)	(27-34)	(24-35)	(20-30)

It is obvious, therefore, that size alone may be unreliable as a specific character, though it can be a guide to identification.

The two species of *Aphelenchoides* herein described as new have been shown to have clear morphological differences. *A. composticola* is also clearly distinguishable from *A. parietinus* by its greater length, smaller relative width and by the number of lines on the lateral field. *A. saprophilus,* however, is extremely close to *A. parietinus.* Although the original population from Docking was 100 μ longer than *A. parietinus* (451 μ), after culture on *Alternaria tenuis* for some months this difference disappeared, as did also an initial difference in the values for *a* (26.3 in *A. parietinus*). The differences which remain are in the shape and curvature of the female tail (Fig. 3), the relative length of the egg, the absence of males in *A. parietinus* and the difficulty of culturing that species on an artificial medium. These are considered sufficient to warrant the designation of *A. saprophilus* as a species.

ZUSAMMENFASSUNG

Aphelenchoides composticola n.sp., eine Ursache für das Versagen der Pilzbeete, ähnelt *A. parietinus,* ist aber länger, relativ schlanker und hat drei Incisuren auf dem Seitenfeld anstatt vier. Männchen sind häufig. *A. saprophilus* n.sp. tritt im Boden und in faulenden Pflanzenteilen auf. Er unterscheidet sich von *A. composticola* durch seine grössere Breite im Vergleich zur Länge, durch vier Längsincisuren und durch die Form der Spicula. Von *A. parietinus* unterscheidet er sich durch die Form des weiblichen Schwanzes, die relative Grösse der Eier, das häufige Auftreten von Männchen und, wie *A. composticola,* durch die Leichtigkeit mit der er auf Agar in Verbindung mit dem Pilz *Alternaria tenuis* kultiviert werden kann.

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BESCHREIBUNG EINIGER NEMATODEN AUS CHAMPIGNONBEETEN [1] [2]

VON

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Bei Untersuchungen des Kompostes von Champignon-Kulturen hinsichtlich seiner Nematodenfauna wurden unter vielen anderen einige charakteristische Fadenwurmarten beobachtet, über die hier berichtet werden soll. Die 7 Arten verteilen sich auf die Unterfamilien *Bunonematinae* (1), *Tylenchinae* (1), *Neotylenchinae* (1) und *Aphelenchinae* (4). Zwei darunter sich befindende noch umstrittene Arten werden zu Vergleichszwecken berücksichtigt. Die 5 restlichen Arten werden im folgenden beschrieben [3].

U. Fam. Bunonematinae

Gattung: *Bunonema* Jägerskjöld 1905

U. Gattung: *Serronema* n. sg.

Genotype: *Serronema dentata* n.sp.

Species typica: *Bunonema* (*Serronema*) *dentata* n.sp. (Abb. 1).

Masse:	5 ♀♀	2 ♂♂
Länge	= 0,24 — 0,29 mm	0,23 — 0,25 mm
Breite	= 0,015— 0,021 „	0,015 „
Ösoph.	= 0,060— 0,081 „	0,060— 0,066 „
Schwanz	= 0,021— 0,024 „	0,030— 0,033 „
a	= 13 —16	15—16
b	= 3,3— 4	3,8
c	= 6 —12	7,5 — 7,6
Vulva	= 50 —57 %	Spic. 21 μ

Mittelwerte:

♀ = Länge = 0,27 mm; a = 14; b = 3,6; c = 9,3; V = 54 %

♂ = Länge = 0,24 mm; a = 15,5; b = 3,8; c = 7,55; Spic 21 μ

Die sehr kleine Art besitzt alle morphologischen Merkmale, die für die Gattung *Bunonema* charakteristisch sind. Kutikula und Kopf sind asymmetrisch ausgebildet. Die rechte Körperseite ist mit einer un-

1) Eingegangen: 17. Juni 1957.

2) Ein Vortrag gleichen Inhalts — durch Lichtbilder unterstützt — wurde am 5.9.1957 auf dem IV. Symposium für Nematologie in Hamburg gehalten.

3) Die Typen befinden sich in der Sammlung der B.Z.A. Naumburg.

paaren, durchgehenden Zapfen- oder Plattenreihe ausgestattet. 2 gestrichelte schmale Membranen verlaufen median. Linke Körperseite mit 5 Kriechleisten. Mundhöhle und Ösophagus sind rhabditoid. Porus und Vulva sowie Anus haben die normale ventrale Lage. Das Charakteristikum und zugleich wichtigste taxonomische Merkmal dieser merkwürdigen Art ist die bereits erwähnte rechtsseitige Zapfenreihe. Die Zapfen sind wie die Zähne eines Sägeblattes angeordnet. Die

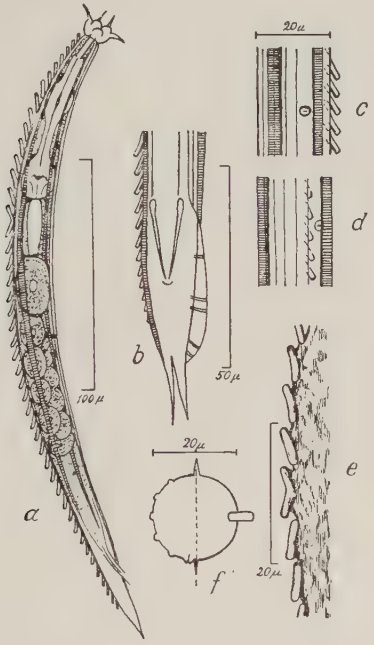

Abb. 1. *Bunonema* (*Serronema*) *dentata* n.sg.n.sp. a Weibchen; b Schwanzende des Männchens; c Ventralansicht, halb schräg gesehen; d Lateralansicht, halb schräg gesehen; e Starke Vergrösserung des Zahnkammes mit Umkehrstelle; f Querschnitt (schemat.).

Zähne werden nach den beiden Körperenden hin kleiner. Im vorderen Körperdrittel sind sie nach vorn gerichtet. Hinter dem Ösophagealbulbus folgt ein doppelzähniges Gebilde, von dem ab die Zapfen nach hinten gerichtet sind. 15-17 Zähne weisen nach vorn, 30-34 nach dem Schwanzende zu. Das Verhältnis 1 : 2 in der Zahnanordnung scheint konstant zu sein. Das männliche Schwanzende trägt eine asymmetrische Bursa. Auf der normal entwickelten linken Seite der leptoderen Bursa findet sich ein breites Velum, das von 5 Papillen gestützt wird, von denen eine präanal steht. Weitere Angaben über morpholo-

gische Einzelheiten, wie z.B. den wirklichen Bau der Zapfen, lassen sich leider nicht machen, da die Tiere bereits präpariert sind und das Präparat mit den wenigen Belegstücken vor Zerstörung bewahrt werden soll. Wegen ihres abweichenden Merkmales liess sich die Art in keine der bekannten Untergattungen einordnen.

Biologie und Ökologie: Von *Serronema dentata* wurden einmal 14 Exemplare (12 ♀♀, 2 ♂♂) in einem Gemisch von Stroh und Sägespänen gefunden, das mit Champignondung vermengt werden sollte. Bei weiteren Untersuchungen des gleichen Substrates konnte die Art nicht wieder aufgefunden werden. Sie scheint selten zu sein. Fundort: Champignonzucht Dieskau/Halle.

U. Fam.: Tylenchinae

Gattung: *Ditylenchus* Filipjev 1934

Ditylenchus sp. (aus Champignonbeeten) (Abb. 2)

oder

Ditylenchus destructor Thorne 1945?

In einer Reihe von Veröffentlichungen über Schadnematoden in Champignonkulturen wurde in den letzten Jahren wiederholt eine als mycelzerstörend bekannte Art unter der Bezeichnung *Ditylenchus* sp. erwähnt. Wegen ihrer Ähnlichkeit mit *Ditylenchus destructor* Thorne, dem Erreger der Älchenkrätze an Kartoffelknollen, erhob sich die Frage, ob sie als selbständige Art zu bewerten oder mit letzterer identisch sei. Neuerdings vertritt man die begründete Ansicht, nach der beide Tiere als Rassen ein und derselben Art aufzufassen sind.

Zum Zwecke weiterer Vergleichsmöglichkeiten seien hier neben einer nochmaligen Abbildung von *Ditylenchus* sp. die Ergebnisse zahlreicher Messungen an Tieren aus Champignonbeeten und von kranken Kartoffelknollen einander gegenübergestellt. Das Untersuchungsmaterial stammt aus dem mitteldeutschen Raume, der Umgebung von Halle und Naumburg/Saale.

Ditylenchus sp. (Champignon) 1956.

Masse:	24 ♀♀	24 ♂♂
Länge	= 1,11 — 1,34 mm	0,81 — 1,02 mm
Breite	= 0,039— 0,048 „	0,024— 0,027 „
Ösoph.	= 0,150— 0,180 „	0,150— 0,174 „
Schwanz	= 0,066— 0,081 „	0,063— 0,066 „
a	= 28 —30	31,2 —38,9
b	= 7,28 — 7,7	5,5 — 6
c	= 17 —18	12,7 —15,2
Vulva	= 83 —84,37 %	Spic. = 21—24 μ
Stachel	= 6,5 — 9 μ	Gub. = 9 μ

Exkretionsporus bei 9,2—13,8 % vom Kopf aus
Ovaranfang „ 13,8 % „ „ „
Testisanfang „ 22 % „ „ „

Mittelwerte:
♀ Länge = 1,27 mm; a = 29; b = 7,4; c = 17; V = 83,9 %
♂ Länge = 0,89 mm; a = 34; b = 5,6; c = 13,5

Ditylenchus destructor Thorne (Kartoffelknollen) 1956

Masse:	12 ♀♀	12 ♂♂
Länge	= 1,20 — 1,40 mm	1,22 — 1,29 mm
Breite	= 0,033— 0,048 „	0,027— 0,034 „
Ösoph.	= 0,150— 0,210 „	0,165— 0,180 „
Schwanz	= 0,078— 0,087 „	0,078— 0,084 „
a	= 28,5 —41,1 „	37 —46,4
b	= 5,95 — 9,06 „	6,96 — 7,8
c	= 15,4 —16,3 „	14,9 —16,3
Vulva	= 81,5 —82,3 %	Spic. = 24—27 μ
Stachel	= 12 μ	Gub. = 9—12 μ

Mittelwerte:
♀ = Länge = 1,306 mm; a = 35,5; b = 7,35; c = 15,9; V = 81,9 %
♂ = Länge = 1,266 mm; a = 41; b = 7,46; c = 15,4; Spic. = 25,2 μ

Hier seien noch die Massangaben aus der Originalbeschreibung von THORNE angeführt.

Ditylenchus destructor Thorne 1945.

♀ = Länge = 0,8-1,4 mm; a = 30-35; b = 8-10; c = 15-20; V = $^{(65)}78\text{-}83^{(10)}$

♂ = Länge = 0,8-1,3 mm; a = 34-40; b = 7-8 ; c = 12-16; T = 73-80

Eine bessere Übersicht dürfte sich aus folgender Nebeneinanderstellung der wichtigsten Daten ergeben.

asse nach Thorne 1945 *Dityl. destr.*	Vom Verfasser 1956 ermittelte Masse *Dityl. destr.* (Kart.)	*Dityl.* sp. (Champignondg.)
= Länge = 0,8— 1,4 mm	1,20— 1,40 (1,306) mm	1,11— 1,34 (1,27) mm
a = 30 —35	28,5 —41,1 (35,5)	28 —30 (29)
b = 8 —10	5,95— 9,06 (7,35)	7,28— 7,7 (7,4)
c = 15 —20	15,4 —16,3 (15,9)	17 —18 (17)
V = 83 %	81,5 —82,3 % (81,9 %)	83,0 —84,37 % (83,9 %)
= Länge = 0,8— 1,3 mm	1,22— 1,29 (1,266) mm	0,81— 1,02 (0,89) mm
a = 34 —40	37 —46,4 (41)	31,2 —38,9 (34)
b = 7 —8	6,96— 7,8 (7,46)	5,5 — 6 (5,6)
c = 12 —16	14,9 —16,3 (15,4)	12,7 —15,2 (13,5)
St. = ?	12 μ	6,5 —9 μ

Hiernach ist *Ditylenchus destructor* 1956 aus Mitteldeutschland in beiden Geschlechtern durchschnittlich grösser und schlanker als der

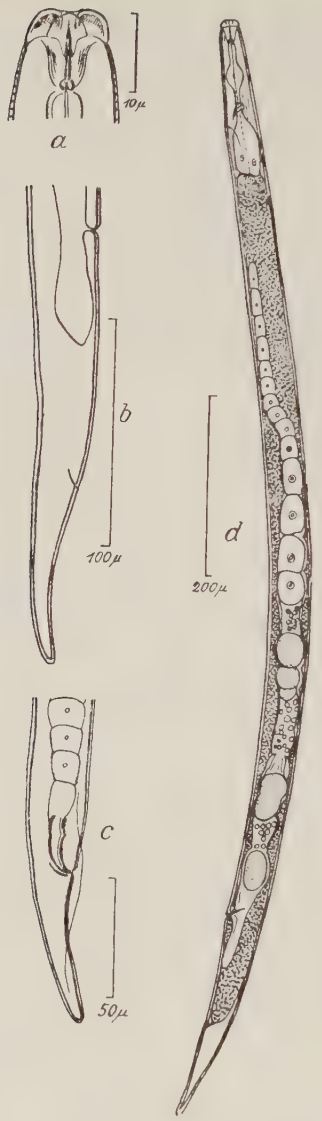

Abb. 2. *Ditylenchus* sp. aus Champignons. a Kopfende; b Schwanz des Weibchens; c Schwanzende des Männchens; d Weibchen.

von THORNE 1945 in den USA beschriebene. Die sich beim Vergleich der Werte für „b" ergebende Abweichung lässt eine grössere Ösophaguslänge bei den 1956 gesammelten Weibchen erkennen. Für die Stachellänge macht THORNE keine Angaben. *Ditylenchus* sp. 1956 ist

durchschnittlich grösser, aber wesentlich plumper als die beiden vorigen. Das vom Wert für „b" Gesagte trifft auch für *Ditylenchus* sp. zu, wo die Abweichung im männlichen Geschlecht besonders auffällig ist. Der grösste Unterschied besteht aber zwischen den Stachellängen der 1956 gemessenen Tiere. Der Stachel des *Ditylenchus destructor* von Kartoffeln ist um $^1/_3$ bis doppelt so lang wie derjenige des an Champignons angetroffenen Parasiten. Diese morphologischen Unterschiede sind doch recht bedeutsam und können erneut zu Zweifeln an der Identität der Arten berechtigen.

Ökologie und Biologie: *Ditylenchus* sp. wurde wiederholt in Champignondünger und an kranken Fruchtkörpern in hoher Individuenzahl festgestellt. *Ditylenchus destructor* trat sehr stark im Herbst 1956 auf einem mit der Sorte „Nova" bestellten Kartoffelacker innerhalb der Naumburger Feldflur auf. Beide Arten bzw. Rassen liessen sich auf sterilem Champignonmycel mit Agarnährboden weiterzüchten.

Fundorte: Champignonzucht Dieskau/Halle und Naumburg/Saale.

Fam.: Neotylenchidae Thorne 1949
U. Fam.: *Neotylenchinae* Thorne 1941
Gattung: *Deladenus* Thorne 1941

Deladenus obesus Thorne 1941 (Abb. 3).

Die bisher nur aus Amerika bekannte Gattung, aus der Rühm 1956 eine neue Art finden konnte, wurde dadurch erstmalig für Europa nachgewiesen. Im gleichen Jahr konnte ich *Deladenus obesus* Thorne 1941 erstmalig für Deutschland nachweisen. Einige Weibchen der Art wurden in Champignonbeeten mit „Perlkrankheit" festgestellt. Männchen wurden nicht gefunden.

	Eigene Masse:	Masse nach Thorne 1941:
♀ =	Länge = 0,97 mm	1,0—1,05 mm
	Breite = 0,053 „	—
	Ösoph. = 0,11 „	—
	Schwanz = 0,03 „	—
	a = 18—19	16—22
	b = 8—9	?
	c = 32	30
	V = 92—93 %	93 %
	Stachel = 0,009 mm	—

Aus der Gegenüberstellung ergibt sich eine gute Übereinstimmung mit den von Thorne 1941 festgestellten relativen Körpermassen, unter denen leider der Wert für „b" fehlt. Der Stachel ist kurz, gedrungen und besitzt mittelgrosse Basalanschwellungen. Unmittelbar hinter dem

Nervenring schiebt sich der Darm in das Metacorpusende des Ösophagus hinein. Der Exkretionsporus mündet kurz hinter dem Nervenring. Die nach vorn ausgestreckte Gonade reicht bis zum Ösophagusende. Ein caudaler Uterussack fehlt. Die Vulva besitzt vorgewölbte Lippen und liegt weniger als 10 % der Gesamtkörperlänge vom Schwanzende aus entfernt. Der postvulvare Körperteil verjüngt sich plötzlich und läuft stumpf keilförmig aus. Die Afteröffnung liegt dicht hinter der

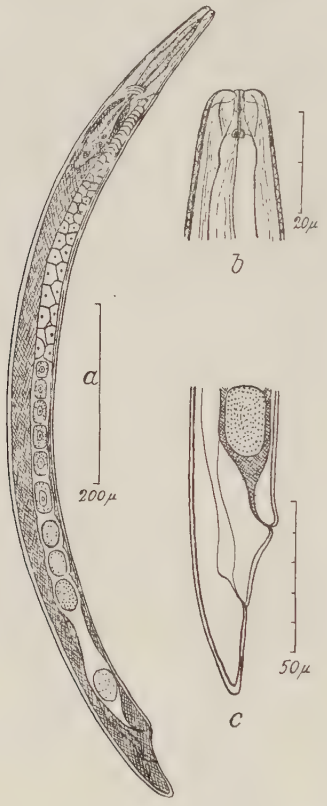

Abb. 3. *Deladenus obesus* Thorne 1941. a Weibchen; b Kopfansicht; c Schwanzende.

Mitte der Strecke Vulva-Schwanzende. Die vorstehenden Angaben entsprechen auch den Hauptgattungsmerkmalen.

Ökologie: *Deladenus obesus* wurde von Thorne 1941 im Gangmulm eines Borkenkäfers an *Abies concolor* gefunden. Saprobe Lebensweise wurde für wahrscheinlich gehalten. In Deutschland 1956 im Dung eines Champignonbeetes festgestellt.

Fundort: Dieskau bei Halle, Champignonzuchtbetrieb. Für Deutschland neu.

FAM.: APHELENCHIDAE STEINER 1949
U. Fam.: *Aphelenchinae* Sch. Stek. u. Teun. 1938
Gattung: *Aphelenchoides* Fischer 1894

Aphelenchoides winchesi Goodey 1927 var. *diversus* n.v. (Abb. 4)

Masse:	6 ♀♀			6 ♂♂		
Länge	= 0,62 — 1,03	(0,76)	mm	0,56 — 0,66	(0,61)	mm
Breite	= 0,024— 0,042	(0,0285)	„	0,021— 0,022	(0,0214)	„
Ösoph.	= 0,063— 0,090	(0,078)	„	0,050— 0,090	(0,0638)	„
Schwanz	= 0,060— 0,091	(0,075)	„	0,040— 0,050	(0,043)	„
a	= 24 —28	(26,5)		26,5 —30	(28)	
b	= 8,5 —11	(9,55)		6,4 —13	(9,8)	
c	= 8,5 —11	(9,25)		13 —15	(14,1)	
V	= 75 —78 %	(76,5 %)		St. = 21—22,5 μ		

Masse von *Aphelenchoides winchesi* Goodey 1927:

♀ = Länge	= 0,80— 0,93 mm	♂ =	0,66— 0,68 mm
a	= 31 —32	a	= 28 —30
b	= —	b	= —
c	= 6,66— 7,1	c	= 10
V	= 70 —83,7 %	St.	= 24—27 μ

Die Variation ähnelt in folgenden Merkmalen *Aphelenchoides winchesi*. In beiden Fällen ist der Kopf deutlich abgesetzt. Der lange und schlanke Stachel hat keine Basalanschwellungen. Exkretionsporus und Nervenring liegen in gleicher Höhe. Ein postvulvarer Uterussack fehlt. Ovar- und Testisenden überschneiden sich mit den Ösophagusdrüsen. Das männliche Schwanzende ist mit 2 Papillenpaaren ausgestattet. Unterschiede ergeben sich bei einem Vergleich folgender Einzelheiten: Die Bulbusform soll nach GOODEY beim Weibchen oval und beim Männchen violinförmig sein. Das trifft für die Variante nicht zu. Auch der Bulbus des weiblichen Tieres ist niemals normal oval ausgebildet. Hunderte von Beobachtungen zeigten stets irgend eine Deformierung desselben. Einige charakteristische Bulbusformen sind aus Abb. 4 ersichtlich. Der Schwanzfaden beim Männchen ist bei der Variation wesentlich kürzer und hat Dolch- oder Dornform. Er ist kürzer als der übrige Schwanzteil. Die hintere Schwanzpapille liegt nicht so weit zurück wie bei *A. winchesi*. Bei einem Vergleich der Masse fällt auf, dass die Variation in beiden Geschlechtern weniger schlank ist und einen viel kürzeren Schwanz hat. Auch der Stachel ist merklich kürzer. Trotz einer gewissen Ähnlichkeit mit *A. winchesi*

Goodey berecntigen m.E. die vorhandenen Unterschiede dazu, die vorliegende Form als var. *diversus* zu bezeichnen.

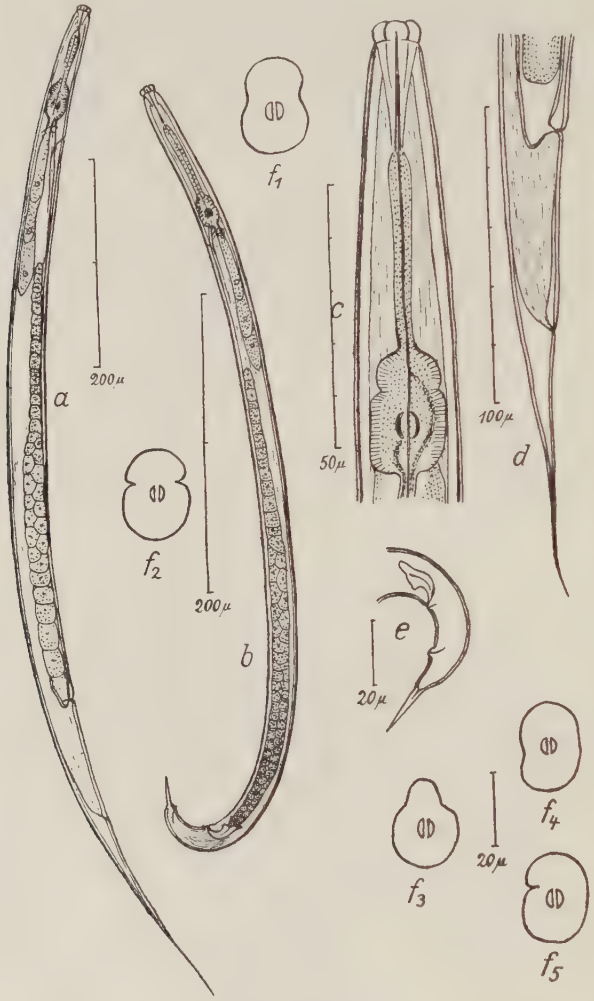

Abb. 4. *Aphelenchoides winchesi* Goodey 1927 var. *diversus* n.v. a Weibchen; b Männchen; c Kopf und Ösophagus; d Schwanz des Weibchens; e Schwanz des Männchens; f_1-f_5 Bulbusformen.

Ökologie: *A. winchesi* Goodey 1927 wurde in Schweinemist in St. Albans (England), erstmalig gefunden. Die var. *diversus* konnte mehrfach in grosser Zahl im Dünger von Champignonbeeten festgestellt werden.

Fundort: Dieskau bei Halle, Champignonzuchtinstitut.

Aphelenchoides oxurus n.sp. (Abb. 5).

Masse:	6 ♀♀	♂
Länge	= 0,55 — 0,66 (0,59) mm	unbekannt
Breite	= 0,024 mm	
Ösoph.	= 0,069— 0,078 (0,075) „	
Schwanz	= 0,036— 0,048 (0,0405) „	
a	= 23 —27 (24)	
b	= 7 — 8,4 (7,8)	
c	= 13 —16 (14,5)	
V	= 79 —82 % (80,2 %)	
St.	= 18 —21 μ (19,5 μ)	

Die gemessenen Tiere stammen aus Champignondung mit „Gipskrankheit". Individuen der gleichen Art, die in stark zersetzten Fruchtkörpern lebten, wichen in den Grössenverhältnissen wesentlich von ersteren ab (Ernährungsbedingte Variabilität). Für sie ergaben sich folgende Masse:

	6 ♀♀	♂
Länge	= 0,972 — 0,975 mm	unbekannt
a	= 28 —29	
b	= 10 —12	
c	= 13 —15	
V	= 79 —80 %	

Auffällige Unterschiede bestehen nur zwischen den Total- und Ösophaguslängen.

Morphologie: Die Lippenregion ist vom Körper deutlich abgesetzt. Der in den hinteren zwei Dritteln stärkere Stachel hat eine fein ausgezogene Spitze. Basalanschwellungen am proximalen Stachelende sind nicht vorhanden. Der fibrilläre Bulbus ist gross und von ovaler Gestalt. An ihn schliesst sich der Darm an. Auf gleicher Höhe liegen auch der Nervenring und der nach vorn mündende Exkretionsporus. Die Kutikula ist fein quergeringelt. Die Vulva ist hinterständig. Ein nach vorn ausgestreckter Gonadenast reicht bis zu den Ösophagusdrüsen. Der caudale Uterussack fehlt. Vor der Vulva wurde ein langes Ei von 90 μ Länge beobachtet. Der kurze Schwanz verläuft konisch und ist scharf zugespitzt, zuweilen ist er in der Mitte etwas eingeschnürt.

Ökologie: *A. oxurus* wurde häufig in Champignondung und an sich zersetzenden Pilzen festgestellt.

Fundort: Champignonzuchtinstitut Dieskau bei Halle.

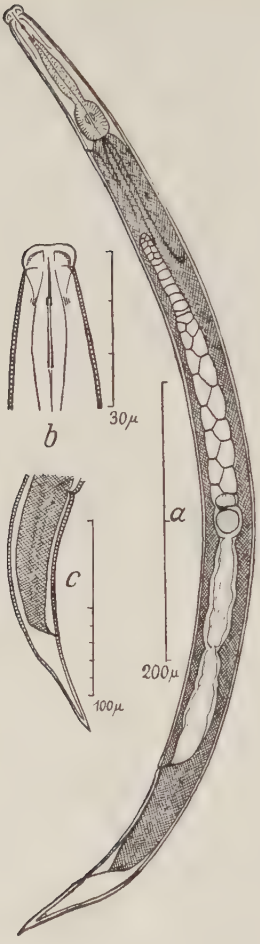

Abb. 5. *Aphelenchoides oxurus* n.sp. a Weibchen; b Kopf; c Schwanzteil.

Aphelenchoides spinosus n.sp. (Abb. 6).

Masse:	8 ♀♀	6 ♂♂
Länge =	0,40— 0,48 mm	0,37— 0,48 mm
a =	22 —24	25
b =	8 —10	10—11
c =	16 —17	20—21
V =	76 —77 %	

Morphologie: Lippenregion deutlich abgesetzt. Stachel klein, Basalanschwellungen schwach, fibrillärer Bulbus auffällig gross, oval und zuweilen eckig, Darmbeginn hinter dem Bulbus. Der Exkretionsporus mündet eine Bulbuslänge hinter dem Bulbusende. Vulva hinter-

ständig. Vorderer Gonadenast nach vorn, bis zu den Ösophagusdrüsen, ausgestreckt. Postvulvarer Ast rudimentär. Schwanz des Weibchens konisch, spitz endigend. Männlicher Schwanz kurz, in einen scharfen, abgesetzten Dorn auslaufend. Kurz vor dem Dornbeginn liegt ein postanales Papillenpaar und ein präanales dicht vor dem Anus. Männliche Gonade gleichfalls weit nach vorn ausgestreckt. Spicula aphelenchoid ohne akzessorisches Stück.

Ökologie: Zahlreiche Individuen dieser ziemlich kleinen Art wurden

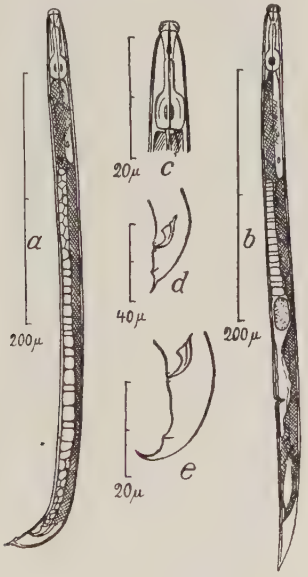

Abb. 6. *Aphelenchoides spinosus*. n.sp. a Männchen; b Weibchen; c Kopfende; d und e Schwanz des Männchens.

in Flachsabfällen festgestellt, die dem Champignondung beigemischt werden sollten.

Fundort: Champignonzuchtinstitut Dieskau bei Halle.

Aphelenchoides cyrtus n.sp. (Abb. 7).

Masse:	6 ♀♀	6 ♂♂
Länge	= 0,50 — 0,57 mm	0,50 — 0,51 mm
Breite	= 0,020— 0,021 „	0,018— 0,019 „
Ösoph.	= 0,06 — 0,07 „	0,06 — 0,07 „
Schwanz	= 0,03 — 0,033 „	0,035— 0,036 „
a	= 24 —28	26 —27
b	= 7,3 — 8	7,4
c	= 16,8	14
V	= 70 —73 %	St. = 10—12 µ

Wie bei den Rhabditiden so gibt es auch bei den Tylenchiden Stellen im System, an denen eine Häufung einander sehr ähnlicher Arten oder Formen auftritt. Oschie (1952) bezeichnet diese Erscheinung als „Formenstau". In solchen Fällen ist die Unterscheidung ähnlicher Formen oft sehr schwierig, wenn nicht gar unmöglich. Formenstau kann auch vorgetäuscht sein, „indem identische Arten verschieden be-

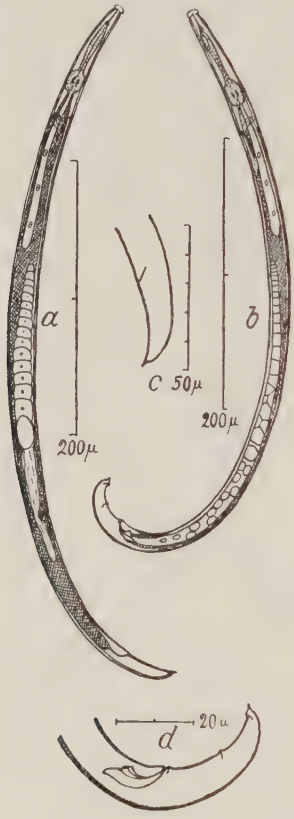

Abb. 7. *Aphelenchoides cyrtus* n.sp. a Weibchen; b Männchen; c Schwanz des Weibchens; d Schwanz des Männchens.

schrieben wurden und noch nicht für synonym erklärt worden sind". Innerhalb der Gattung *Aphelenchoides* bietet sich ein charakteristisches Beispiel für Formenstau, wenn es sich um *A. parietinus* (Bastian 1865) Steiner 1932 handelt. Schon Micoletzky (1914) gliedert die sehr veränderliche Art in eine grössere Zahl von Formen und Subformen auf. Goodey (1951) gibt eine Liste von 13 Synonyma der

Art. Franklin (1955) beschreibt *A. parietinus* Bastian 1865 aus *Xanthoria parietina* neu und stellt in 2 Tabellen die Indizes von *A. parietinus* der verschiedenen Autoren denen der verwandten Arten gegenüber. Dabei wird auf die Möglichkeit verwiesen, dass sich unter den für synonym erklärten Arten doch noch selbständige Arten mit geringen Unterschieden befinden können. Dieser Ansicht von Franklin muss ich mich anschliessen. Auf Grund folgender Merkmale halte ich *A. cyrtus* für eine gute Art.

Diagnose: Grosse Ähnlichkeit mit den bisher als *A. parietinus* beschriebenen Arten besteht. Übereinstimmungen finden sich im Bau des Kopfes und des Ösophagus, in den Schwanzformen, in der Anordnung der Schwanzpapillen beim Männchen und im Bau und der Ausbreitung der Gonaden. Der 10-12 μ lange Stachel ist bei der vorliegenden Art deutlich geknöpft. Der Nervenring liegt dicht hinter dem Bulbus. Eine Bulbuslänge hinter dem Bulbusende mündet der Exkretionsporus. Geschlechterverhältnis 1 : 1. In der Wärmestarre krümmen sich beide Geschlechter stets bogenförmig und können infolge dieses Verhaltens immer von anderen Aphelenchoiden getrennt werden, bei denen die Weibchen mehr gestreckt bleiben und nur das männliche Schwanzende stark eingekrümmt wird. Bei einem Vergleich mit dem von Franklin neubeschriebenen *A. parietinus* Bastian zeigt sich, dass der Stachel dieser Art weniger deutlich geknöpft ist, der Exkretionsporus unmittelbar am Bulbusende liegt, die Vulva weiter nach vorn verlagert und der Schwanz länger ist. Ferner sind keine Männchen der Art bekannt. Der von Goodey beschriebene *A. parietinus* ist grösser und schlanker, der Ösophagus ist kürzer und der Schwanz des Weibchens länger. Die Mündung des Exkretionsporus liegt 2 Bulbuslängen hinter dem Bulbusende. Auf die Beschreibung von Steiner (1932) trifft das Gleiche zu. In seiner Zeichnung liegt der Exkretionsporus in Höhe des hinteren Bulbusabschnittes. Von einem postvulvaren Uterussack erwähnt Steiner nichts. Neben den bestehenden morphologischen Unterschieden scheint mir jedoch die Krümmungsform von *A. cyrtus* in der Wärmestarre ein wichtiges Artmerkmal zu sein. Die Erscheinung, die bei verwandten Formen nicht zu beobachten ist, kann nur auf einen anderen anatomischen Bau dieser Art zurückzuführen sein.

Die aufgezeigten Unterschiede dürften m.E. ausreichen, *A. cyrtus* als selbständige Art anzusehen.

Ökologie: *A. cyrtus* wurde mehrfach in den Lagern von Erdflechten

und in Champignondünger mit Flachs- und Strohbeimengung beobachtet.

Fundort: Siebengebirge, Saaletal und Champignonzuchtinstitut Dieskau bei Halle.

SUMMARY

A new subgenus, 4 new species of nematodes and a new variation of the superfamilies *Rhabditoidea, Tylenchoidea* and *Aphelenchoidea* are described. Because it is new for Germany, *Deladenus obesus* Thorne 1941 is redescribed. In a confrontation of *Ditylenchus destructor* Thorne 1945 from potatoes and *Ditylenchus* sp. from mushrooms (Champignons) the existing morphological differences are demonstrated. The nematodes come from beds of the Champignonzuchtinstitut Dieskau near Halle and from a potatofield near Naumburg/Saale.

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DISKUSSION

J. H. Schuurmans Stekhoven: Zu *Aphelenchoides winchesi* Goodey var. *diversus* (Abb. 4) möchte ich bemerken, dass man im allgemeinen vermeiden sollte von Varietäten zu sprechen, wenn gute und feste Unterschiede gefunden werden. Statt von einer Varietät zu sprechen, würde ich im vorliegenden Falle vorschlagen, diese als neue Art *Aphelenchoides diversus* P. zu bezeichnen.

Fr. Paesler: Da, wie ich sehe, die vorhandenen morphologischen Unterschiede als Voraussetzung für die Aufstellung einer neuen Art für ausreichend gehalten werden, akzeptiere ich den Vorschlag. Die Variante wird in *Aphelenchoides diversus* n. sp. umbenannt.

Nematologica II (1957) : 329-334. — Leiden, E. J. Brill

TYLENCHULUS MANGENOTI N. SP. (NEMATODA-TYLENCHULIDAE) [1])

PAR

MICHEL LUC

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Des pieds de *Dorstenia embergeri* G. Mangenot, petite Moracée assez rare en Côte d'Ivoire, avaient été repiqués sous ombrière à l'Institut d'Enseignement et de Recherches Tropicales. Quelques mois plus tard ils souffraient d'une chlorose généralisée, les feuilles, beaucoup plus pâles que normalement, présentant en sus des taches jaunâtres, diffuses, parfois assez étendues. Le port lui-même est affecté, la plante restant petite, les feuilles moins développées.

Un examen du système souterrain a révélé la présence sur le rhizome et les racines d'un très grand nombre d'individus d'une nouvelle espèce de *Tylenchulus*. Cette espèce est également présente sur les *Dorstenia* de la station naturelle, mais en quantité plus faible; corrélativement les symptômes de chlorose sont assez peu marqués.

Les femelles de *Tylenchulus mangenoti* n.sp. sont enfoncées par leur extrémité antérieure dans les racines et le rhizome, la partie postérieure du corps restant libre. Chaque femelle est isolée et s'entoure d'une sorte de coque („matrix") brunâtre, dure, pouvant atteindre jusqu'à près de 400 μ, grossièrement sphérique et contenant de nombreux œufs et larves. Les fragments de sol n'adhèrent pas à ces coques qui sont lisses, brillantes et facilement repérables.

Les examens et les mensurations ont été effectués, pour les femelles, sur des exemplaires vivants, ce qui est permis par le peu de mouvements qu'elles présentent, la queue seule se mouvant de façon appréciable. Pour les larves et le mâle, des racines et des fragments de rhizome ont été placés dans un extracteur de Seinhorst et les individus récoltés tués (à 65° pendant une minute) puis fixés au F A 4 : 10. Des examens complémentaires ont porté sur des individus colorés in situ par le lactophénol à la fuchsine acide et montés dans le lactophénol simple.

1) Accepté : le 1 Juillet 1957.

Tylenchulus mangenoti n.sp.

Femelles: L = 0,330-0,410 mm a = 2.5-2,8
b = 2,5 -2,9 c = 14,8-16
V = 71-87 % P.E. [1]) = 50-61 %

„Corps de forme très typique, en croissant presque entièrement refermé sur lui-même, à extrémités très amincies et centre élargi et épaissi, s'inscrivant presque parfaitement dans l'espace compris entre un grand cercle de 125-155 μ de diamètre et un cercle tangent intérieur de diamètre $^1/_3$. (Du fait de cette particulière configuration les coefficients donnés ci-dessus n'ont qu'une relative valeur). Partie antérieure, enfoncée dans les tissus de l'hôte, de forme très variable: tantôt courte et trapue, tantôt très étirée. Lèvres formant un anneau continu peu élevé. Sclérotisation céphalique fine, en anneau légèrement épaissi à la base. Stylet de 13-15 μ, le protorhabdion faisant à lui seul les $^2/_3$ de la longueur; boutons basaux hémisphériques, lisses. Guide annulaire très fin, situé un peu au-dessus de la moitié du stylet. Muscles protracteurs du stylet à peine indiqués. Glande oesophagienne antérieure débouchant à 6-8 μ en dessous du stylet. Partie antérieure de l'oesophage cylindrique, faisant place insensiblement à un bulbe médian pyriforme très développé de 25-29 μ de diamètre; isthme étroit (4-5 μ), court (15-18 μ), entouré presque immédiatement sous le bulbe par un anneau nerveux peu développé; bulbe basal glandulaire sphérique comportant deux parties avec chacune un noyau le divisant en deux hémisphères à peu près égaux. Junction oesophago-intestinale non visible. Intestin lui-même non discernable, sauf à la partie postérieure qui est suivie d'un rectum assez long aboutissant à un anus très peu visible mais rencontré chez toutes les femelles examinées. Pore excréteur situé à 50-61 % de la longueur totale avec une ouverture large. Canal excréteur se dirigent généralement vers l'arrière mais parfois aussi vers l'avant. Ovaire unique très long faisant de nombreuses circonvolutions; ovocytes sur un seul rang sauf dans la zone de multiplication. Utérus volumineux, à paroi épaisse, musculaire. Pas de spermathèque individualisée, les spermatozoïdes, agglomérés en boule, se localisant dans la partie postérieure de l'utérus. Pas de membranes paravulvaires („*flaps*"). Sac post-utérin absent. Cuticule très mince à la partie antérieure du corps située dans les tissus de l'hôte; très épaissie dans la partie libre du corps, jusqu'à atteindre 4-5 μ dans la

1) Pore excréteur.

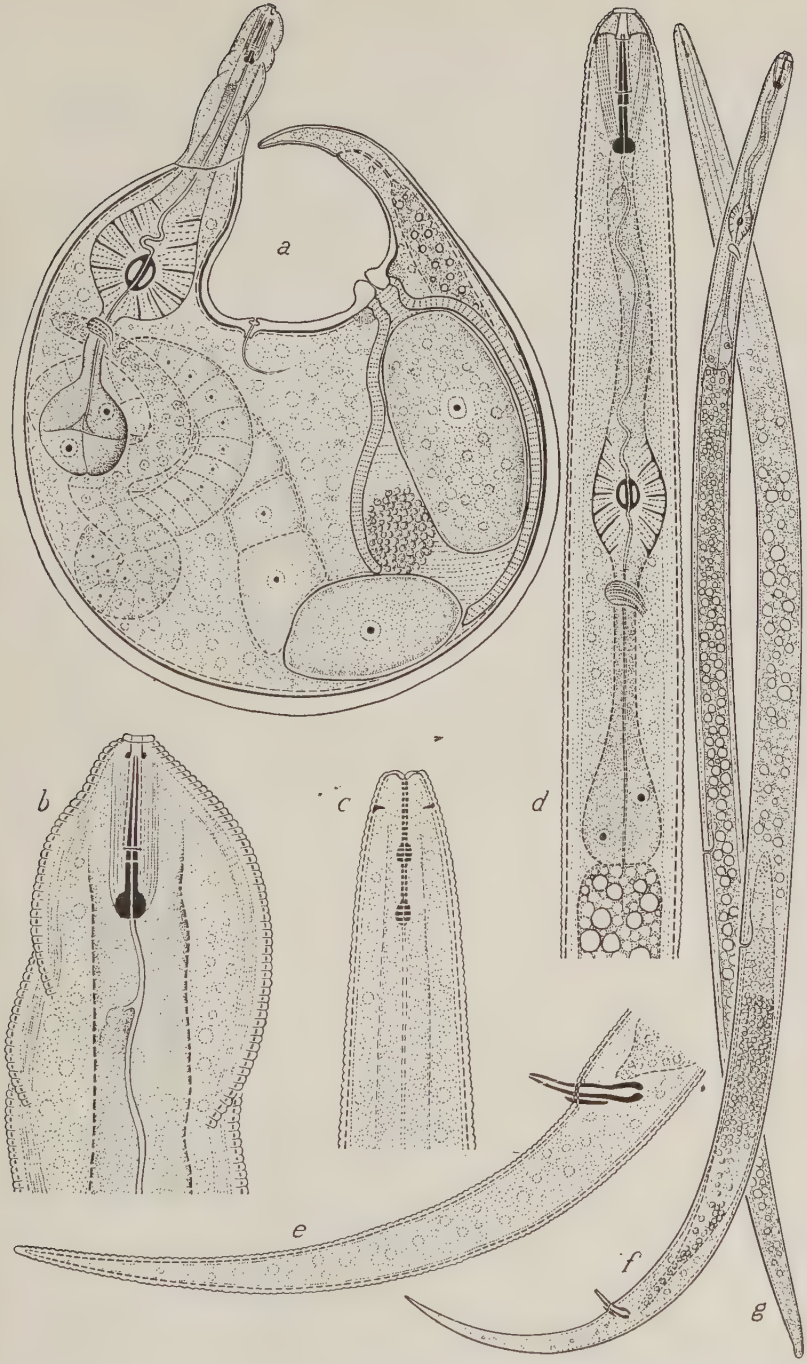

Fig. 1. *Tylenchulus mangenoti* n. sp. Femelle: a: vue totale; b: partie antérieure. Mâle: f: vue totale; c: partie antérieure; e: partie postérieure. Juvénile; g: vue totale; d: partie antérieure. (Gross. : a, f, g, = × 570. b., c, d, e, = × 1000).

partie centrale du corps. Phasmides, deirides, hémizonide, champ latéral non observés. Œufs gros: 59-64 × 29-32 μ; chaque thèque entourant une femelle en comprenant environ une centaine. Femelles fécondées avant leur pleine maturité."

Mâle: L = 0,326 mm a = 27,1 b = ?
c = 6,5 T = 32 % P.E. = 61,6 %

Parmi plusieurs centaines d'individus juvéniles extraits au Seinhorst nous n'avons rencontré qu'un seul mâle. Ils sont donc considérablement plus rares que chez *T. semi-penetrans* Cobb, 1913.

„Corps fin, cylindrique, aminci vers l'avant et régulièrement effilé vers l'arrière. Partie antérieure atrophiée. Lèvres non marquées. Anneau sclérotisé très fin visible postérieurement. Stylet dégénéré; on distingue seulement une sclérotisation de 13,5 μ de long, comportant deux renflements, mais sans lumière visible. Limites oesophagiennes ainsi que la lumière perceptibles sur quelque distance; postérieurement l'oesophage est dégénéré. Testicule unique de 95 μ de long. Spicules faiblement arqués, de 13,5 μ de long, à extrémité proximale renflée. Gubernaculum simple, droit, mince, de 4 μ de long. Ni gaine spiculaire ni bourse. Corps rétréci au niveau des spicules. Queue longuement et régulièrement effilée, à extrémité pointue. Cuticule marquée de stries fines (0,7 μ). Champ latéral avec 2 incisures, de 1,4 μ de large. Deirides, phasmides et hémizonide non observés. Pore excréteur à 61 % de la longueur du corps, un peu au-dessous de l'extrémité de la gonade. Canal excréteur dirigé vers l'avant."

Juvéniles: L = 0,276-0,287 mm a = 24,1-26,5 b = 3,5-4,2
c = ? P.E. = 57-62 %

„Corps cylindrique, peu effilé vers l'avant, s'amincissant régulièrement vers l'arrière. Cuticule finement striée présentant un champ latéral d'environ 1,5 μ de large, avec 2 incisures. Lèvres soudées en un anneau étroit. Sclérotisations céphaliques fines mais nettes. Stylet de 12,4-14 μ de long, à protorhabdion faisant des $^3/_5$ aux $^2/_3$ de la longueur totale. Boutons basaux hémisphériques. Glande oesophagienne antérieure s'ouvrant à 5-6 μ en-dessous du stylet. Guide du stylet très fin, situé à mi-hauteur. Oesophage à procorpus cylindrique; corpus bien développé, elliptique, avec valvules sclérotisées importantes; isthme étroit suivi d'un bulbe basal bien individualisé, renflé en poire, comportant deux noyaux visibles. Anneau nerveux situé immédiatement sous le corpus. Intestin contenant de gros granules. Anus non observé. Queue effilée, arrondie à l'extrémité."

Diagnose: *Tylenchulus mangenoti* n. sp. diffère de *T. semi-penetrans* Cobb, 1913 par les caractères suivants: (1) forme et dimensions de la femelle, (2) présence chez la femelle d'une queue plus développée, (3) mâle avec une partie antérieure de l'oesophage plus dégénérée et une queue plus longue et plus effilée.

Hôte type: *Dorstenia embergeri* G. Mangenot (Moraceae): racines et rhizome.

Localité type: Institut d'Enseignement et de Recherches Tropicales d'Adiopodoumé, I.D.E.R.T. près Abidjan. Côte d'Ivoire.

Holotype: Femelle. Tube N° A 26. Laboratoire de Nématologie. I.D.E.R.T. Abidjan.

Allotype. Mâle. Tube N° 22. Laboratoire de Nématologie. I.D.E.R.T. Abidjan.

La description de cette espèce confirme les vues de Raski (1957) sur l'appartenance des deux genres *Tylenchulus* Cobb, 1913 et *Trophotylenchulus* Raski, 1957 à une même sous-famille et rapproche leur parenté. En effet chez les femelles et les juvéniles de *Tylenchulus mangenoti* n.sp. les lèvres, soudées, forment un anneau bas mais net, très voisin de ce que Raski décrit chez *Trophotylenchulus floridensis* Raski, 1957 et considère comme caractéristique du genre. Cependant le pore excréteur situé très postérieurement, l'absence de gaine spiculaire, permettent de ranger plus logiquement l'espèce décrite dans le genre *Tylenchulus* Cobb, 1913.

Un point à considérer est la présence chez les femelles de l'espèce décrite d'un anus peu visible mais fonctionnel, ainsi que nous avons pu le constater en pressant des femelles vivantes entre lame et lamelle. Chez toutes les autres espèces appartenant à la famille des *Tylenchulidae* l'anus de la femelle est décrit comme apparemment non fonctionnel (*Tylenchulus semi-penetrans* Cobb, 1913; *Trophonema arenarium* (Raski, 1956) Raski 1957, *Trophotylenchulus floridensis* Raski, 1957) ou n'a pas été remarqué (*Sphaeronema californicum* Raski & Sher, 1952). Chez ces espèces, comme chez *Tylenchulus mangenoti* n.sp., le nombre d'œufs produits par une femelle est souvent très grand; cela nécessite un courant de substance nutritive assez fort ce à quoi correspond bien la partie antérieure du tube digestif très développée. Dans le cas d'un anus non fonctionnel, l'évacuation des déchets devrait se faire soit à travers la cuticule, souvent épaisse, soit uniquement par le canal excréteur; ces deux solutions semblent problématiques. Il nous semble plus logique d'admettre que chez les

Tylenchulidae, les femelles possèdent un anus souvent difficilement discernable, surtout sur des échantillons fixés, mais néanmoins fonctionnel.

SUMMARY

The description of *Tylenchulus mangenoti* n. sp. confirms the opinion of Raski (1957) on the close relations between the genera *Tylenchulus* Cobb, 1913 and *Trophotylenchulus* Raski, 1957. The presence of a functional anus is noted in the females of *Tylenchulus mangenoti* and the author suggests that the anus and the posterior part of the alimentary canal, though difficult to perceive in the females of others *Tylenchulidae*, may nevertheless be functional.

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Nematologica II (1957) : 335. — Leiden, E. J. Brill

SCIENTIFIC NOTES

J. H. Schuurmans Stekhoven[1]) : *A note on the peculiar feeding habits of Telorhabditis longispina* (Reiter)

Prof. Dr. José M. Cei, Santiago de Chile, found that in his cultures eggs of the Leptodactylid frog, *Eusophus taeniatus* Girard, were completely destroyed by a nematode. The frog develops directly in a gelatinous egg capsule and abounds in woods of *Drymis* and *Notophagus,* trees commonly occurring in the cold regions of South Chile (Valdivia) and less commonly around Santiago.

Slides sent contained ten females and a number of larvae, mostly in the first stage, which apparently had recently escaped from the uteri of their mothers. Reproduction, therefore may have been hermaphroditic or parthenogenetic. The structure and dimensions of the worms agreed with those of *Telorhabditis longispina* (Reiter 1928), the triangular distribution of teeth in the metastome, the form of the tail and the coarsely annulated cuticle being identical with Reiter's figure. The vulva was slightly anterior to the middle of the body, the tail elongate and gradually tapering to a fine point. *Telorhabditis longispina* is a cosmopolitan saprobiont living in such media as cow dung. This is the first record of occurrence in frogs' eggs. Several specimens were found inside the gelatinous material of the egg envelope.

Reiter, G. (1952). Zur Systematik und Oekologie der zweigeschlechtlichen Rhabditiden. *Arb. zool. Inst. Univ. Innsbruck,* **3**, 93-184.

Francis Hemming[2]) : *Colloquium on Zoological Nomenclature to be held in London in July, 1958 in connection with the Fifteenth International Congress of Zoology.*

It is hoped that at the Fifteenth International Congress of Zoology to be held in London in July, 1958 it will be possible for that Congress finally to approve and adopt the new text of the International Code for Zoological Nomenclature as revised by the preceding Congresses held in Paris in 1948 and Copenhagen in 1953. As arranged at the Copenhagen Congress, a draft of the revised text will be submittted for this purpose to the London Congress.

In order to assist the Congress in the examination of the draft of the revised Code and to provide ample opportunity for the discussion of the questions involved, arrangements have been made between the Secretariat-General of the Congress and the International Trust for Zoological Nomenclature under which a Colloquium on Zoological Nomenclature is being organised by the International Trust on the lines of the Colloquium organised by that body in connection with the Copenhagen Congress.

The Colloquium will open on Wednesday, the 9th July i.e. one week prior to the opening of the London Congress. It is hoped that this body will be able to relieve the Congress of the bulk of the work involved in the scrutiny of the draft of the revised Code and will be able to submit to the Congress agreed recommendations as to the text to be adopted. Invitations to the Colloquium have already been issued by the Trust to a large number of zoologists known to be interested

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1) Biological Laboratory, Noury and Van der Lande, Deventer.

2) Managing Director and Secretary, International Trust for Zoological Nomenclature and Secretary to the International Commission on Zoological Nomenclature.

in zoological nomenclature. In addition, arrangements have been made between the Trust and the Secretariat-General of the Congress under which an invitation to the Colloquium will be issued by the Trust to any member of the Congress who expresses a desire to take part in its discussions but who has not as yet received a separate invitation.

NEMATOLOGICAL NEWS

Thanks are due to Dr. A. H. Meyl for the news column and bibliography which appeared in earlier issues. The Editors regret that these items will not be included in future volumes. Dr. Meyl wishes to thank all colleagues for their co-operation and to say that both the news and the bibliography may be continued elsewhere.

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" , " II : May 23rd, 1957
" , " II : September 10th, 1957
" , " II : December 5th, 1957

Nematologica II, Suppl. (1957): 337-341 S — E. J. Brill, Leiden

ERÖFFNUNGSANSPRACHE

VON

H. GOFFART
Biologische Bundesanstalt, Inst. f. Hackfruchtbau, Münster, Westf., Deutschland

Meine sehr verehrten Damen und Herren!

Ich habe die Ehre, das 4. Internationale Symposium für Nematologie zu eröffnen. Ich begrüsse Sie alle auf das herzlichste. Mein Gruss gilt zunächst den Kollegen aus dem mitteldeutschen Raum, die sich trotz mancher Schwierigkeiten in stattlicher Anzahl hier eingefunden haben. Ebenso herzlich begrüsse ich die Kolleginnen und Kollegen aus dem Ausland, die den oft weiten Weg nicht gescheut haben und danke ihnen für ihr Erscheinen. Ganz besonderen Dank möchte ich Ihnen, Herr Professor Dr. MEVIUS, abstatten, der Sie uns entgegenkommender Weise die Institutsräume für unser Symposium zur Verfügung gestellt und diesen schönen Hörsaal so hübsch von gärtnerischer Seite haben ausgestalten lassen. Bedauerlicherweise ist es Professor PETERS, London wegen Erkrankung nicht möglich, am Symposium teilzunehmen und den Vorsitz der ersten Sitzung zu übernehmen. Wir haben ihm brieflich unsere besten Wünsche für baldige Genesung ausgesprochen.

Meine Damen und Herren!

Auf der letzten Tagung in Wageningen wurde beschlossen, das 4. Symposium 1957 in Hamburg abzuhalten. Es steht dieses Mal im Schatten des in der nächsten Woche hier beginnenden 4. Internationalen Pflanzenschutzkongresses. Viele von Ihnen werden auch diesen Kongress besuchen, daher hielten wir es für zweckmässig, Hamburg als Tagungsort des Symposiums zu wählen. Dass auf dem Pflanzenschutzkongress auch eine Sektion für pflanzenparasitische Nematoden besteht, die an zwei Tagen ihre Sitzungen abhält, mag vielleicht als Nachteil für unser Symposium erscheinen. Da beide Tagungen aber in Personalunion miteinander verbunden sind und eine sachliche Trennung der Probleme vorgenommen wurde, glaube ich, dass eine Überschneidung weitgehend vermieden wird.

Das diesjährige Symposium wird erstmalig von der Society of European Nematologists getragen, die 1955 in Wageningen gegründet

wurde. Der geschäftsführende Ausschuss dieser Gesellschaft hat im Oktober 1956 beschlossen, auf diesem Symposium 4 Problemstellungen herauszugreifen, nämlich Taxonomie, Stengelälchen, Wurzelgallenälchen und wandernde Wurzelnematoden. Er hofft dadurch, für die Diskussionen etwas mehr Zeit zu erübrigen.

Es ist das erste Mal, dass das Internationale Symposium für Nematologie in Deutschland tagt. Gestatten Sie mir daher, dass ich mit einigen Worten auf die Entwicklung der Nematodenforschung in Deutschland eingehe.

Erste Ansätze einer wissenschaftlichen Untersuchung pflanzenparasitischer Nematoden finden wir bereits zu Beginn des 19. Jahrhunderts in der Veröffentlichung von Steinbuch über Gräserälchen (1799). Der Beginn einer eigentlichen Nematodenforschung setzt in Deutschland dann um die Mitte des 19. Jahrhunderts ein. 1958 werden 100 Jahre vergangen sein, seitdem Kühn in der Zeitschrift für wissenschaftliche Zoologie seine erste Veröffentlichung über das Auftreten von Stengelälchen an *Dipsacus fullonum* herausbrachte. Fast um die gleiche Zeit (1860) beobachtete Schacht einen Schädling an Rüben, der nach seinen Forschungen ein Fadenwurm war. Einige Jahre später (1871) stellte A. Schmidt fest, dass dieser Fadenwurm die Ursache der sogenannten „Rübenmüdigkeit" ist. Zu Ehren seines Entdeckers nannte er ihn *Heterodera schachtii*. Der Rübennematode rief zur damaligen Zeit namentlich in Mitteldeutschland bedeutsame wirtschaftliche Verluste im Zuckerrübenbau hervor. Nach Jablonowski (1909) mussten bis 1876 allein 24 Zuckerfabriken ihren Betrieb einstellen, weil nicht genügend Rüben zur Verarbeitung bereit standen. Das Rübennematodenproblem stand auch in den folgenden Jahrzehnten im Mittelpunkt des Interesses. Das geht nicht nur aus den Veröffentlichungen von Kühn, Liebscher, Hollrung und Strubell hervor, sondern ergibt sich auch aus der von Julius Kühn veranlassten Gründung einer Versuchsstation für Nematodenvertilgung im Jahre 1889, der ersten phytopathologischen Station im damaligen Deutschen Reiche.

Wenn auch durch radikale Fruchtfolgemassnahmen in den folgenden Jahren die schlimmsten Ausfälle unterbunden werden konnten, so ist das Rübennematodenproblem seitdem doch nicht aus dem Blickpunkt der Forschung entschwunden. Es wurden im Gegenteil immer wieder neue wertvolle Erkenntnisse gesammelt. Erinnert sei nur an die Untersuchungen von Baunacke über das Problem der Aktivierung der Rübennematodenlarven und an die Veröffentlichung von O. Schmidt, der die Unterschiede von Rüben- und Hafernematoden herausgestellt

hat. Die zweite Hälfte des vorigen Jahrhunderts hat uns auch auf anderen Gebieten der Nematodenforschung die ersten Früchte gebracht. Es war die Zeit, in der CLAUS über in Humus auftretende Älchen berichtete (1863), SCHNEIDERS Monographie der Nematoden erschien (1866) und BÜTSCHLI seine grundlegenden Untersuchungen über freilebende Nematoden herausbrachte (1873). Auf dem Gebiet der Phytonematologie erschienen die Veröffentlichungen von GREEFF (1864 und 1872), MÜLLER (1884) und FRANK (1885) über Auftreten, Biologie und phytopathologische Bedeutung des Wurzelgallenälchens, das unter dem Namen *Heterodera radicicola* zunächst in die Wissenschaft einging und heute eine eigene aus mehreren Arten bestehende Gattung darstellt, die z.Z. wieder das besondere Interesse der Taxonomen unter den Nematologen besitzt.

Zu Beginn des zwanzigsten Jahrhunderts trat MARCINOWSKI mit einem Beitrag zur Biologie und Morphologie von *Cephalobus elongatus* und *Rhabditis brevispina* (1906), ferner mit einer Veröffentlichung über Blattälchen (1908), vor allem aber mit ihrer ausgezeichneten Zusammenfassung über parasitisch und semiparasitisch an Pflanzen lebende Nematoden hervor (1909), die sehr viele eigene Beobachtungen enthält. Kleinere Untersuchungen von SCHWARTZ über Blattälchen schlossen sich an (1913). Erneut wurde die Aufmerksamkeit auf die Nematoden durch die bereits erwähnte Veröffentlichung von BAUNACKE gelenkt (1922), die in der Folgezeit eine ganze Anzahl von Arbeiten zur Frage des Aktivierungsproblems ausgelöst hat (NEBEL, MOLZ, GOFFART, RADEMACHER u.a.).

In den gleichen Zeitraum fallen auch die ersten Untersuchungen über den Kartoffelnematoden, der zwar schon 1913 von ZIMMERMANN in der Umgebung von Rostock beobachtet, aber erst 1923 von WOLLENWEBER als selbständige Art beschrieben wurde. Der Kartoffelnematode hat seitdem von Jahr zu Jahr an Bedeutung zugenommen und sein Verbreitungsareal vergrössert. Er ist zu einem Quarantäneschädling ersten Ranges geworden und steht heute im Brennpunkt internationaler Erörterungen.

Dass in den letzten 30 Jahren auch Untersuchungen über Hafernematoden (*Heterodera major* = *H. avenae*), Erbsennematoden (*Heterodera göttingiana*) und Kleenematoden (*Heterodera trifolii*), über Stengelälchen (*Ditylenchus dipsaci*), *Pratylenchus pratensis*, Blattälchen und andere Nematodenarten durchgeführt und Bekämpfungsmöglichkeiten erarbeitet werden konnten, sei nur am Rande vermerkt.

In neuester Zeit befasst man sich in Deutschland in erheblichem Um-

fange auch mit den wandernden Wurzelnematoden als Erreger von Wachstumsstörungen und Müdigkeitserscheinungen (SWART-FÜCHTBAUER, 1954, FISCHER, 1955, WEISCHER, 1956, NOLTE und DIETER, 1957). Erste Ergebnisse liegen auch auf diesem Gebiet vor.

Einen bedeutsamen Aufschwung hat schliesslich die Entwicklung nematizider Mittel genommen, seitdem sich auch die deutsche chemische Industrie der Nematodenfrage angenommen hat. Während wir noch vor 20 Jahren über kein hinreichend wirksames nematizides Mittel verfügten, und selbst der Schwefelkohlenstoff als Nematizid mehr eine theoretische als eine praktische Bedeutung besass, gelang es der Industrie inzwischen unter Berücksichtigung der im Auslande gemachten Erfahrungen Stoffe zu entwickeln, die eine sehr hohe nematizide Wirkung besitzen. Wenn diese auch noch nicht allen Wünschen entsprechen, so sind die Fortschritte auf diesem Sektor der Nematodenforschung doch unverkennbar.

Wichtig erscheint mir, dass die Nematodenforschung nicht mehr eine rein deskriptive Wissenschaft ist, sondern sich von der Fläche der Mannigfaltigkeiten mehr und mehr auch in die Tiefe der Gesetzmässigkeiten versenkt und sich den komplexen Problemen zuwendet. Dieser Grundzug ist heute in allen Ländern zu beobachten, in denen Nematologen tätig sind. Er führt, wenn ihm ein Erfolg beschieden sein soll, zwangsläufig zu einer engen Zusammenarbeit zwischen Nematologen mit Entomologen, Mykologen, Virologen, Pflanzenphysiologen, Bodenkundlern und Biochemikern. Ein solches „Team-work" wird auch nicht auf einzelne Länder beschränkt bleiben. Da sich die Nematoden ebensowenig wie andere Schädlinge an politische Grenzen halten und der moderne Verkehr von Land zu Land, ja von Kontinent zu Kontinent sich in wenigen Stunden abspielt, ist auf dem Gebiete der Nematodenforschung eine internationale Zusammenarbeit unerlässlich. Ich darf es als ein glückliches Zeichen ansehen, dass wir seit 1955 die Society of European Nematologists haben, in der jede auf dem Gebiet der Nematologie tätige Person willkommen ist. Als ein zweites die Völker verbindendes Glied sehe ich die Zeitschrift „Nematologica" an, die in den bisher erschienenen Heften den Beweis für eine fruchtbare wissenschaftliche Zusammenarbeit bereits erbracht hat. Eine solche Zusammenarbeit kann nur gedeihen, wenn sie auch gepflegt wird. Daher unser Bestreben: nach Möglichkeit jedes zweite Jahr zu einem Erfahrungsaustausch einmal zusammenzukommen. Dass auch dieses Symposium dem Gedankenaustausch förderlich sein und die Kollegen einander näher bringen möge, ist mein aufrichtiger Wunsch.

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Nematologica II, Suppl. (1957) : 342-346 S — E. J. Brill, Leiden

DAS VORKOMMEN VON ARTGEMISCHEN BEI PFLANZENPARASITÄREN NEMATODEN

VON

M. OOSTENBRINK

Plantenziektenkundige Dienst, Wageningen

Es ist bekannt, dass in Kulturböden normalerweise verschiedene Gattungen von Nematoden neben- und durcheinander vorkommen. Hinsichtlich der Pflanzenparasiten ist dies in neueren Veröffentlichungen betont geworden (Steiner 1953; Oostenbrink, s'Jacob & Kuiper 1956). Weniger bekannt ist die Tatsache, dass von jeder Gattung am gleichen Orte und in derselben Bodenprobe oft auch mehr als eine Art auftreten. Diese Sachlage hat die taxonomischen und ökologischen Resultate mancher früherer Untersuchungen ungünstig beeinflusst oder sogar entwertet; sie verursacht auch jetzt noch Schwierigkeiten, wenn man mit Gattungen arbeitet, von denen nur ein kleiner Teil der Arten beschrieben ist. In diesem Vortrag werden einige Erfahrungen mitgeteilt über das Vorkommen von Artgemischen bei den Gattungen *Heterodera, Pratylenchus, Paratylenchus, Tylenchorhynchus, Rotylenchus* und *Meloidogyne.*

Heterodera-Arten sind im allgemeinen spezialisierte Parasiten, die nur bei einseitigem Anbau von gewissen Wirtspflanzen merkbare Populationen aufbauen. Verschiedene Arten sind in geringen Konzentrationen weit verbreitet. Ackerland enthält öfters eine, nicht selten zwei und bisweilen drei Arten. Dies ist offenbar in verschiedenen westeuropäischen Ländern bekannt, dank der ausgedehnten Bodenuntersuchungen über das Auftreten von Kartoffelnematoden, die nebenbei auch Daten über andere *Heterodera*-Arten ergaben.

Auffällig ist das Auftreten von *Heterodera*-Gemischen in Wiesen. Eine Überprüfung in den Niederlanden zeigte, dass in alten Wiesen das Vorkommen von zwei oder drei Arten in 70% der Felder normal ist. Nur ausnahmsweise (3% der Felder) wurden keine Zysten gefunden (siehe Tabelle I). Auf die ätiologische und wirtschaftliche Bedeutung dieser Arten hinsichtlich der Wüchsigkeit von Gras und Klee wird an anderer Stelle eingegangen werden.

Einige Arten der Gattung *Pratylenchus* kommen in bestimmten Ge-

Tabelle I

Das Vorkommen von gemischten Populationen von Heterodera-Arten nach Untersuchungen von 661 alten Wiesen in den Niederlanden in 1952.

— = keine Zysten p = *H. punctata* Thorne [1])
a = *H. avenae* Mort., Rostr. et K. Ravn = *H. major* O. Schmidt [1])
t = *H. trifolii* Goffart [1]) Undet. = Zysten nicht determiniert.

Zahl der untersuchten Parzellen	Zahl der *Heterodera*-Zysten in 100 ml Erde	Zahl der Parzellen mit								Undet.
		—	p	a	t	p + a	p + t	a + t	p + a + t	
661	0-126, durchschn. 19	21	47	23	50	67	221	41	127	64

1) Die Andeutungen sind sensu lato zu betrachten. Es gibt Anzeichen für alle drei Arten, dass sie nicht in jeder Hinsicht mit den ursprünglichen, als Parasiten von Ackerbaugewächsen beschriebenen Arten identisch sind.

genden so allgemein und weit verbreitet vor, dass man eine andere Art kaum studieren kann, ohne ihnen zu begegnen. Das gilt z.B. für *P. pratensis* (de Man) Filipjev in vielen leichteren und für *P. minyus* Sher et Allen (sensu lato) in vielen schwereren Böden.

In einer früheren Arbeit wurde *P. penetrans* (Cobb) Sher et Allen in zwei auch mit *pratensis* infizierten Feldern studiert (Oostenbrink, 1956). Die Populationen beider Arten reagierten unabhängig voneinander auf die angebauten Gewächse. Rüben drückten die Populationen von *penetrans* und *pratensis,* Kartoffeln aber nur die von *pratensis.* Für *penetrans*-empfindliche Gewächse war Rübe eine viel bessere Vorfrucht als Kartoffel, was den geringen *penetrans*-Zahlen im Boden und in den Wurzeln der nachgebauten Kontrollpflanzen entsprach.

Dasselbe wurde in einem Feld mit *P. penetrans* in einer anderen Gegend festgestellt. Nach zweijährigem Anbau auf denselben Parzellen waren die *Pratylenchus*-Zahlen im Boden nach Rüben wider alles Erwarten noch sehr hoch. Analyse der *Pratylenchus*-Population zeigte, dass speziell in den Rübenparzellen nicht mehr *penetrans,* sondern eine Art der *minyus*-Gruppe in den Vordergrund getreten war und dass in demselben Feld auch *pratensis* als dritte *Pratylenchus*-Art anwesend war. Ein zweijähriger Anbau von Rüben hatte die Population signifikant geändert. Ausserdem war, gerade durch die Rüben, eine *Paratylenchus*-Population aufgebaut, die sehr viel höher lag als dies nach

anderen Gewächsen der Fall war. Diese Beispiele weisen auf eine starke Dynamik der *Pratylenchus*- und auch *Paratylenchus*-Populationen hin (siehe Tabelle II).

Tabelle II

Einfluss verschiedener Gewächse auf die Zusammensetzung einer gemischten Pratylenchus - Population.

Gewächse in 1955 und 1956	*Pratylenchus* (*penetrans* / *pratensis* / *minyus*) pro 200 ml Erde im Frühjahr 1957	
Beta vulgaris L.	530	(0 / 0 / 20)
Solanum tuberosum L.	300	(20 / 0 / 0)
Avena sativa L.	725	(18 / 2 / 0)
Trifolium pratense L.	635	(12 / 8 / 0)

Von *Paratylenchus* werden öfters Gemische angetroffen, die unbeschriebene, nicht gut charakterisierte Taxa enthalten, was die Arbeit mit dieser Gruppe erschwert. In einer vorläufigen Übersicht wurden neben 3 beschriebenen Arten 10 andere Formen gesammelt, von denen die meisten wahrscheinlich gute Arten sind.

Ein kompliziertes *Paratylenchus*-Gemisch wurde schon früher analysiert (Oostenbrink, 1954). Hierbei wurden in demselben Grasfeld vier Taxa konstatiert, die in anderen Fällen auch unabhängig voneinander gefunden wurden.

Seitdem sind, besonders in Wiesen, verschiedene Gemische von zwei oder drei gut zu unterschiedende Taxa aufgefunden worden. Auch in Obstgärten auf schweren Böden wurden in verschiedenen Fällen Gemische von zwei verschiedenen Formen festgestellt.

Tylenchorhynchus enthält viele Arten, die ebenfalls komplizierte Gemische bilden. Die Arten sind relativ gross und gut zu erkennen, dank einer neueren Revision der Gattung (Allen, 1955). Hierbei wurde die Zahl der Arten von 12 auf 34 gebracht. In den Niederlanden stieg die Zahl von 4 auf 11; ausserdem sind noch einige unbeschrieben.

Arten, die in Bauland in verschiedenen Feldern durcheinander vorkommen, sind z.B. *T. dubius* (Bütschli) Filipjev und *macrurus* (Goodey)Filipjev. In Wiesen kommen vielerlei Gemische vor, z.B. von *dubius, maximus* Allen und *nanus* Allen, von *dubius, maximus* und *brevidens* Allen, von *dubius, brevidens* und *tessellatus* J. B. Goodey und von *maximus, ornatus* Allen und *obscurus* Allen.

Für *Rotylenchus* gilt ungefähr dasselbe wie für *Tylenchorhynchus*.

Diese Gattung enthält auch viele grosse und gut erkennbare Arten, aber die Gruppe ist sehr unvollständig bearbeitet und dadurch wenig zugänglich.

Artgemische kommen öfters vor. In diesem Zusammenhang kann auf ein historisches Gemisch unter dem Namen *Rotylenchus robustus* (de Man) Filipjev hingewiesen werden. De Man hat *Tylenchus robustus* in 1876 und 1880 beschrieben und in 1884 eine Zeichnung einer anderen Art hinzugefügt. Seitdem wusste niemand mehr, was *Tylenchus robustus,* Genotype der Gattung *Rotylenchus* Filipjev 1936, war, bis rezente Untersuchungen der alten Sammlung von de Man hier Klarheit verschafften (siehe Loof & Oostenbrink, 1958).

Ob Gemische von *Meloidogyne*-Arten öfters vorkommen, ist zur Zeit nicht zu beurteilen. Die Identifikation der einzelnen Arten ist erst seit kurzem bekannt und stützt sich auf variable morphologische Merkmale, sodass man geneigt ist, abweichende Stücke an erster Stelle als Varianten innerhalb der Art zu betrachten.

Im folgenden Fall aber wurde mit Sicherheit ein Artgemisch festgestellt. In einem mit *M. incognita* (Kofoid et White) Chitwood verseuchtem Glashaus wurden gegen diesen Nematoden resistente Tomaten, *Lycopersicum peruvianum* (L.) Mill., aus Samen gezüchtet, die dennoch Befallssymptome an den Wurzeln zeigten. Identifikation der Nematoden und die Gallen zeigten, dass *M. hapla* Chitwood vorlag, sodass es sich hier offenbar um zwei *Meloidogyne*-Arten in demselben Boden handelte.

Die genannten Daten geben nur eine unvollständige Darstellung; sie lassen aber den Schluss zu, dass Artgemische bei vielen wichtigen Gattungen von phytophagen Nematoden öfters vorkommen, und dass die Zusammensetzung der Mischpopulationen sich schnell ändern kann. Bei taxonomischen und ökologischen Untersuchungen von natürlichen Populationen wird man hiermit im allgemeinen zu rechnen haben.

SUMMARY

The occurrence of mixtures of two or more species of a genus at the same place is illustrated for six genera of plant parasitic nematodes. The frequency of such mixtures and their dynamic character under the influence of cropping is stressed.

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DISKUSSION

H. GOFFART: Wir fanden im Gebiet des Niederrheins oft auf Kleefeldern *Heterodera trifolii* und *Meloidogyne hapla* zusammen. In einigen Fällen wurde auch Kleekrebs, *Sclerotina trifoliorum* Erikss. in Verbindung mit den genannten Nematoden gefunden. Die Berater beachten hauptsächlich den Befall durch Kleekrebs, aber wir meinen, dass die genannten Nematoden vielfach wichtiger sind.

M. OOSTENBRINK: Neben der schon genannten *Heterodera trifolii* sind nach unseren Erfahrungen *Meloidogyne hapla* und auch *Pratylenchus*-Arten wichtige Schädlinge von Klee, besonders auch von Weissklee in Wiesen. Bei Schlechtwüchsigkeit von Klee und dem Verschwinden des Weissklees aus vielen Wiesen spielen sie eine wichtige Rolle. Kleekrebs ist nach holländischen Erfahrungen nicht von grosser Bedeutung als Schädling von Weissklee in Wiesen; Rotklee kann stark unter diesem Parasit zu leiden haben.

E. B. BROWN: *Meloidogyne hapla* in Great Britain is mainly a pest in horticultural nurseries where perennial plants are grown. On farm land is it usually only a pest after a field has grown lucerne for several years or where brassicas, legumes, sugar beet, carrots etc. have been grown for a number of years in the absence of cereals. A 1-year cereal break seems to be sufficient to reduce the *M. hapla* population to a very low level, unless there is a high population of weeds.

M. OOSTENBRINK: We have exactly the same experiences in the Netherlands. I can add the observation, that legumes are very efficient host plants and that for that reason meadows (clover!) on light soils normally show up a marked infestation by *M. hapla*. When all fields of such a farm are sampled one can normally recognize meadows on the presence of the *Meloidogyne* larvae (though there are other indicator nematode species as well).

J. H. SCHUURMANS STEKHOVEN: bemerkt, dass die Taxonomie der pflanzenparasitären Nematoden nicht solche Schwierigkeiten liefert, wie die der verschiedenen freilebenden Nematoden, da es leichter ist, die Larven auf die entsprechenden Arten zu beziehen. So wurde z.B. der marine Nematode *Ascolaimus elongatus* (Bütschli) Ditlevsen in der Literatur zu 6 verschiedenen Arten und 3 Genera gerechnet, weil man die verschiedenen Stadia unabhängig voneinander untersucht hatte.

Nematologica II, Suppl. (1957): 347 S – - E. J. Brill, Leiden.

ÜBER EINIGE IN CHAMPIGNONKULTUREN GEFUNDENE NEMATODEN AUS DEN GATTUNGEN *BUNONEMA, DITYLENCHUS, DELADENUS* UND *APHELENCHOIDES*

VON

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ZUSAMMENFASSUNG [1])

An Hand von Diapositiven wird über taxonomische Merkmale einiger in Champignondünger festgestellter Nematoden berichtet. Zuerst wird mit der neu aufgefundenen Untergattung *Serronema* — Gattg.: *Bunonema* — (Genotype *S. dentata* n. sp.) bekannt gemacht. — Durch Gegenüberstellung von *Ditylenchus destructor* Thorne 1945 mit Tieren der gleichen Art aus Mitteldeutschland a) von Kartoffeln und b) von Champignons wird auf die bestehenden Unterschiede hingewiesen. — In Champignonbeeten mit „Perlkrankheit" wurden einige weibliche Tiere eines Nematoden gefunden, die mit *Deladenus obesus* Thorne 1941 übereinstimmen. Die Art wurde 1956 erstmalig in Deutschland festgestellt. — Die Merkmale einer Variation von *Aphelenchoides winchesi* Goodey 1927, die u. a. niemals einen normal gebauten Bulbus besitzt, werden gezeigt. Die var. *diversus* wurde wiederholt in verschiedenen Zuchtsubstraten festgestellt. Weibliche Tiere einer mir aus der Literatur nicht bekannt gewordenen *Aphelenchoides*art werden als *Aphelenchoides oxurus* n. sp. vorgestellt. Die Tiere wurden in Dünger und an zerfallenden Pilzen angetroffen. — An Flachsabfällen wurde eine kleine *Aphelenchoides*art festgestellt. Sie wurde *Aphelenchoides spinosus* n. sp. genannt. Der kurze männliche Schwanz endigt in einem abgesetzten, scharfen Dorn.

Zu einem umstritteten Objekt ist *Aphelenchoides parietinus* geworden. Hier liegt ein Beispiel für Formenstau innerhalb der Gattung vor. Als sehr veränderliche Art bekannt, gliedert sie schon Micoletzky (1914) in eine grössere Zahl von Formen und Subformen auf. Goodey (1951) gibt eine Liste von 13 Synonyma der Art. M. T. Franklin (1955) beschreibt *A. parietinus* Bastian aus der Flechte *Xanthoria parietina* neu und stellt in 2 Tabellen die Indizes von *A. parietinus* der verschiedenen Autoren denen der verwandten Arten gegenüber. Dabei wird auf die Möglichkeit verwiesen, dass sich unter den für synonym erklärten Arten doch noch selbständige Arten mit geringen Unterschieden befinden können. Als Beispiel hierzu Bilder und Daten der von mir neu aufgestellten Art *Aphelenchoides cyrtus* und einiger anderer fraglicher Arten, die *A. parietinus* nahe stehen.

1) Die vollständige Arbeit ist in *Nematologica* **2** (1957): 314-328 erschienen.

Nematologica II, Suppl. (1957): 348 S — E. J. Brill, Leiden

WAS IST *APHELENCHUS NEGLECTUS* RENSCH?

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ZUSAMMENFASSUNG

Im Jahre 1924 beschrieb RENSCH eine neue pflanzenparasitäre Nematodenart unter dem Namen *Aphelenchus neglectus*. Wie STEINER 1927 aber zeigte, gehört diese Art zur Gattung *Pratylenchus*; sie wurde von ihm synonymisiert mit *P. pratensis* (DE MAN). Diese Auffassung ist von allen späteren Autoren beibehalten worden. Erneute Untersuchungen an Material von den von RENSCH erwähnten Fundstellen in der Nähe von Magdeburg und Halle, zusammen mit einigen Daten aus den Beschreibungen von RENSCH und GOFFART (1927) zeigten aber, dass die RENSCH'sche Art nicht synonym zu *pratensis* DE MAN ist, sondern tatsächlich eine neue Art darstellt. Sie unterscheidet sich von *pratensis* durch den Bau des Schwanzes, den plumperen Körper und die aus nur 2 Ringen zusammengesetzte Lippengegend. Weiterhin tritt *neglectus* auf lehmhaltigem Boden auf, während *pratensis* leichtere Böden bevorzugt.

Pratylenchus neglectus (RENSCH 1924) n. comb., wie die Art also heissen muss, steht morphologisch *P. steineri* LORDELLO, ZAMITH und BOOCK 1954 und *P. minyus* SHER und ALLEN 1953 sehr nahe. Es ist nicht ganz ausgeschlossen, dass *minyus* und *neglectus* nur Formen einer Art darstellen; Versuche hierüber sind im Gange.

Die Art ist in Deutschland und den Niederlanden verbreitet und vielleicht schädlich an Getreidearten und anderen Gewächsen.

Über diese Untersuchungen wird an einer anderen Stelle ausführlich berichtet werden.

DISKUSSION

J. H. SCHUURMANS STEKHOVEN bemerkt, dass die Rensch'sche Figur den Eindruck eines Weibchens macht, das in der Haut der letzten Larve steckt.

P. LOOF: In der Tat ist die äussere Körperwand in Höhe der Vulva nicht unterbrochen. Die typische Schwanzform findet sich aber auch bei völlig erwachsenen Exemplaren und stellt somit kein Häutungs-Artefakt dar.

H. GOFFART: Vor 30 Jahren kannten wir noch nicht die feineren morphologischen Unterscheidungsmerkmale bei den *Pratylenchus*-Arten. Bemerken möchte ich, dass mir auch Material von Salzmünde vorgelegen hat, aber hier fand ich nur sehr selten Männchen.

P. LOOF: Offenbar haben Sie ein ähnliches Artengemisch vor sich gehabt wie in den Tabellen gezeigt wurde. Die einzige der darin enthaltenen Arten, die Männchen besitzt, nämlich *P. penetrans*, war nur sehr spärlich vertreten. Die Übereinstimmung unserer Ergebnisse ist also recht gut.

Nematologica II, Suppl. (1957) : 349-354 S. — E. J. Brill, Leiden

NEMATODEN UND BIOLOGISCHE BEKÄMPFUNG DER INSEKTEN

VON

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Nachdem die Grenzen und Gefahren der chemischen Bekämpfung erkannt worden waren, hat in den letzten Jahren die biologische Schädlingsbekämpfung in zahlreichen Ländern wieder erheblich an Bedeutung gewonnen. Die Untersuchungen konzentrieren sich vor allem auf Virosen, Bakteriosen, Mykosen, die Räuber- und Schlupfwespenfauna der verschiedensten Schadinsekten. Gerade in neuerer Zeit konnten in Nordamerika auf dem Gebiete der biologischen Schädlingsbekämpfung durch die nachträgliche Einfuhr von Nützlingen, die Kombination chemischer und biologischer Methoden und durch die mikrobiologische Bekämpfung beachtliche Erfolge erzielt werden.

Wie nun aus mehreren Arbeiten hervorgeht, sind auch zahlreiche Insektenarten, darunter wirtschaftlich bedeutende Schädlinge, mit adult- und larvalparasitischen Nematodenarten vergesellschaftet. Zweifellos ist heute, gemessen an der Zahl der noch zu erwartenden insektenparasitischen Nematoden, nur eine geringe Anzahl bekannt. Die Coleopteren stellen das Hauptkontingent der Wirte. Dagegen wurden in anderen Insektengruppen (z. B. Lepidopteren) bisher noch keine oder vereinzelt parasitische Nematodenarten nachgewiesen.

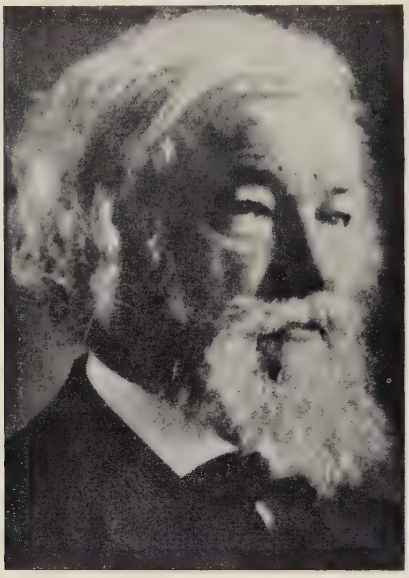
Die zum Teil starke Infizierung der Populationen verschiedener Arten mit parasitischen Nematoden, die dadurch bedingte verminderte Fertilität bzw. Sterilität oder sogar Kastration der befallenen Wirtsindividuen veranlasste bereits vor vielen Jahren einige Autoren, wie z. B. Fuchs (1915-1938), eine biologische Bekämpfung von Insekten durch Nematoden zu diskutieren. Fuchs war in seinen Äusserungen optimistisch und glaubte, dass in dem einen oder anderen Fall sogar Scolytiden erfolgreich bekämpft werden könnten. Spätere an Erdflöhen mit *Howardula phyllotretae* Oldham 1933 und an *Popillia japonica* Newman mit *Neoaplectana glaseri* Steiner 1929 durchgeführte Bekämpfungsversuche (Glaser et al. 1932-1942) scheiterten oder brachten keinen anhaltenden Erfolg. Weitere beachtenswerte Ver-

suche mit parasitischen Nematoden, wurden dann erst in letzter Zeit wieder in Angriff genommen (RÜHM 1954-1956; DUTKY & HAUGH 1955; FRANZ 1957). So erscheint es angebracht, der Frage nachzugehen, ob nicht insbesondere durch eine Verbesserung der Züchtungsmethoden Misserfolge in der Bekämpfung vermieden werden und so die insektenparasitischen Nematoden als biologisches Kampfmittel in verstärktem Masse Verwendung finden können.

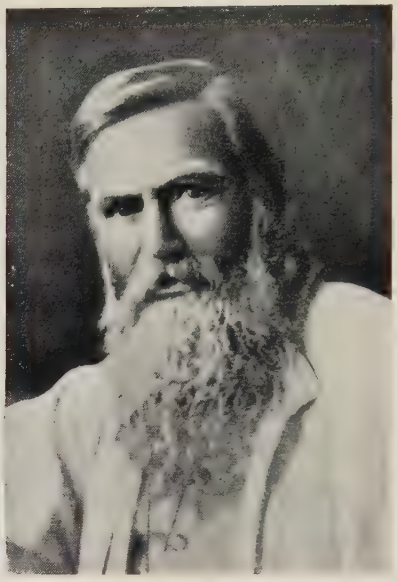
Die Mehrzahl der parasitischen, mit Insekten vergesellschafteten Nematoden gehört systematisch zu den Tylenchida THORNE 1949. Der Entwicklungszyklus dieser Adult- und Larvalparasiten, den ich kurz erwähnen möchte, verläuft bei vielen Arten, grob gesehen, gleichartig. Im erstgenannten Fall produziert das adulte, in der Leibeshöhle parasitisch lebende Weibchen Larven. Diese wandern meist nach 2 Häutungen aus dem Enddarm oder den Geschlechtswegen der Wirte aus und werden nach 1-2 weiteren Häutungen geschlechtsreif. Die Weibchen werden, obwohl ihre Gonadenanlage klein und unscheinbar ist, frühzeitig begattet. Erst das begattete Weibchen sucht wieder einen neuen Wirt auf, in dem es sich mit oder ohne Häutungen unter mehr oder minder starker Veränderung des Wurmkörpers zum Adultparasiten entwickelt.

Bei den Larvalparasiten befinden sich lediglich Larven in den Wirtstieren. Sie häuten sich in den Wirten meist einmal und werden freilebend nach einer weiteren Häutung geschlechtsreif. Die aus den Eiern der begatteten Weibchen schlüpfenden Larven wandern sofort wieder in Wirte ein. Demnach bilden diese zu den Tylenchida gehörigen Adult- und Larvalparasiten stets eine einzige „freilebende Generation" aus. Da der Entwicklungszyklus der einzelnen Arten streng fixiert ist, besteht keine Möglichkeit, die freilebenden Geschlechtstiere in einem Zuchtmedium zur Vermehrung zu bringen bzw. mehrere „Generationen" heranzuzüchten, um eine möglichst grosse Anzahl infektionsbereiter Nematodenweibchen zu erhalten. Wir können nur die in der Leibeshöhle eines Wirtes befindlichen bzw. auswandernden Larven zu freilebenden Geschlechtstieren züchten. Daher ist eine jeweils stark infizierte Wirtspopulation die Voraussetzung für die Gewinnung einer genügend grossen Anzahl von Nematodenweibchen bzw. Larven, um ihre auf dem Infektionswege einsetzende, starke Verminderung auszugleichen bzw. eine erhöhte Infizierung einer Wirtspopulation zu erreichen. Da aber zahlreiche Umweltfaktoren (rasche Entwicklung des Wirtes, Feuchtigkeit etc.), die nicht im einzelnen erörtert werden können, die Beziehungen Wirt-Parasit besonders bei einer Massen-

Teilnehmer des 4. Internationalen Symposium für Nematologie in Hamburg



Julius Kühn
1825—1910



Otto Bütschli
1848—1920
(*Aus Buddenbrook: Biologische Grundprobleme und ihre Meister*, 1951)

H. W. Wollenweber
1879—1949

W. Baunacke
1883—1934

vermehrung zuungunsten der parasitischen Nematoden beeinflussen, ist allein wegen des Entwicklungszyklus ein erfolgreicher und wirtschaftlich tragbarer Einsatz der parasitischen Tylenchida in der biologischen Schädlingsbekämpfung nicht zu erwarten.

Hinzu kommt, dass der Einfluss der insektenparasitischen Tylenchida auf den Einzelwirt wie die Wirtspopulationen im allgemeinen gering ist. Selten führen sie den Tod ihrer Wirte herbei. Vorwiegend können wir eine verminderte Fertilität, selten eine weitgehende Sterilität oder Kastration beobachten. In stark infizierten Populationen des zu den Scolytiden gehörigen *Myelophilus piniperda* L. erwiesen sich beispielsweise 4 %, in denen des *Ips acuminatus* 7 %, des *Scolytus scolytus* Fabr. 8 % und des *Pityokteines curvidens* Germ. 14 % der Individuen als steril. Bei den anderen Insektenarten ist der Prozentsatz steriler Individuen in den meisten Fällen geringer als bei den Scolytiden. Weitere Untersuchungen ergaben, dass nicht einmal die Lebensdauer infizierter gegenüber nicht infizierter Käfer wesentlich verkürzt ist. Parasitierte Weibchen des Buchdruckers, *Ips typographus* L., waren sogar in der Lage, nach den „normalen" Bruten sog. Geschwisterbruten anzulegen. Lediglich bei Einwirkung weiterer ungünstiger Umweltfaktoren (z. B. in Laboratoriumsversuchen) auf die parasitierten Wirte kam es zu stärkeren Ausfällen und zu einer erhöhten Mortalität innerhalb der betreffenden Populationen. Bei Mischinfektionen durch verschiedene Arten bzw. bei einer grösseren Parasitengarnitur ist die Möglichkeit einer nachhaltigen Beeinflussung der Wirte und Populationen gegeben (z. B. bei *Hylastes ater* Payk. mit allein 4 verschiedenen adultparasitischen Arten). In keinem Falle konnte bisher bei den Tylenchida-Parasiten eine Massenvermehrung beobachtet werden, die zum Zusammenbruch einer Insektenpopulation geführt hätte. Die insektenparasitischen Tylenchida führen ihr Dasein während ihrer Entwicklung auf Kosten ihrer Wirte, ohne diese zu töten oder wesentlich zu schädigen. Sterilisation und sonstige stärkere Schädigungen bilden vielfach Ausnahmeerscheinungen, die man nicht überbewerten darf. Die phylogenetischen Beziehungen zwischen den Tylenchida-Parasiten und ihren Wirten sind meist relativ alt, so dass sich ein Gleichgewicht eingestellt hat, das selten eine Störung erfährt.

Weiterhin darf man nicht ausser acht lassen, dass die Tylenchida-Parasiten weitgehend wirtsspezifisch und somit eng an die Lebensweise ihrer Wirte gebunden sind. Daher scheidet auch eine Infizierung anderer, parasitenfreier Wirte aus. Schliesslich ist bei einer grossen Anzahl Insekten mit mehr oder minder verborgener Lebensweise, z.B. bei den Ipiden, Cerambyciden, Curculioniden, die Infektion bzw. das

Einführen der begatteten Nematodenweibchen in den jeweiligen Lebensraum (Brutgänge!) ausserordentlich schwierig. Die Verluste wären selbst unter der Voraussetzung günstiger Zuchtbedingungen viel zu hoch, um ein wirtschaftlich tragbares Verfahren zu ermöglichen.

Erfolgsaussichten für die Verwendung parasitischer Nematoden zur biologischen Bekämpfung bestehen nur dort, wo mehrere „Generationen" im oder am Wirt oder freilebend durchlaufen werden und die Voraussetzung für eine Massenvermehrung gegeben ist. In solchen Fällen kann auch durch Verbesserung der Zuchtmethoden eine möglichst grosse Anzahl infektionsbereiter Stadien unabhängig von der Entwicklung des Wirtes und der Jahreszeit gewonnen werden. Ferner ist es günstig, wenn die freilebenden Stadien, Larven oder Adulte zur Anabiose befähigt sind und so ungünstige Milieubedingungen bis zur Infektion des Wirtes überbrückt werden können.

Innerhalb der Tylenchida sind mir bisher nur die mit Langrüsslern vergesellschafteten *Stictylus*-Arten bekannt geworden, die mehrere Generationen ausbilden. Die freilebenden Geschlechtstiere dieser *Sphaerularia*-artigen Adultparasiten nähren sich vorwiegend von Pilzen aus den Frassgängen ihrer Wirte. Die infektionsbereiten, teilweise zur Anabiose befähigten Entwicklungsstadien entstehen fortlaufend. Ihre Züchtung bereitet keine grösseren Schwierigkeiten. Wenn diese spezifischen *Stictylus*-Arten für die biologische Bekämpfung ihrer Wirte nicht in Frage kommen, so lassen diese Funde doch hoffen, dass wir auch innerhalb der Tylenchida einmal Arten finden werden, die für eine biologische Schädlingsbekämpfung in Frage kommen.

Die besten Aussichten für eine biologische Bekämpfung von Schadinsekten durch Nematoden bestehen bei den weniger wirtsspezifischen Steinernematiden, wie dies schon die früheren Versuche von Glaser erkennen lassen. Neuerdings wurde in den USA festgestellt, dass eine dieser Arten Bakterien in den Darm der Wirte einschleppt, die dann durch die sich entwickelnden Bakteriosen zugrundegehen. In den getöteten Insekten vermehren sich dann die Nematoden, die eine grössere Anzahl „Generationen" erzeugen. Diese Nematoden-Bakterien-Krankheit könnte einmal praxisreif werden, zumal die erfolgreiche Massenzucht bereits gelang. Auch bei einigen nicht freilebenden Rhabditiden und Diplogasteriden, die ebenfalls Bakterien einschleppen können, bestehen in dieser Hinsicht gewisse Erfolgsaussichten. Gegebenenfalls wird man damit rechnen müssen, die in Frage kommende Schädlingspopulationen mehrmals mit Nematoden zu infizieren, um die durch Umwelteinflüsse eingetretenen Verminderungen an Parasiten

auszugleichen. Durch eine solches Verfahren würde dann auf keinen Fall eine Störung der Biozönose mit unangenehmen Nebenerscheinungen, wie sie leider bei chemischen Bekämpfungen verschiedentlich auftreten können, verursacht.

Abschliessend müssen wir doch feststellen, dass die parasitischen Nematoden in der biologischen Schädlingsbekämpfung, auch dann, wenn es gelingt, in den günstig gelagerten Fällen die Zuchtmethoden zu verbessern, eine verhältnismässig geringe Rolle spielen werden.

SUMMARY

The question whether the failure of nematodes to control noxious insects could be overcome by improved techniques was discussed and whether nematodes in greatly increased numbers could find a use as agents of biological control.

The adult and larval parasitic nematodes belonging to the Tylenchida which form the majority of insect parasitic species are largely excluded as they have a fixed cycle of development and produce only a single freeliving generation. It is not possible to get them to multiply on culture media. Since many factors influence unfavourably the relationship between host and parasite, especially in attempting to obtain great increase, the successful and practical introduction of these Tylenchida is not to be expected because of the nature of the development cycle. In general also the influence of Tylenchid parasites on their hosts and host populations is small. Their host specificity also excludes use to infect other hosts.

Prospects of success are greater where more generations are passed through either in the host or in the freeliving stage, thus giving the prerequisite for successful mass breeding. Furthermore the outlook is favourable if the freeliving stage is capable of anabiosis so that adverse conditions can be endured until the host is again infected. Within the Tylenchida only the species of *Stictylus*, associated with weevils, produce more freeliving generations but because they are host specific they are largely excluded from biological control. The best prospects occur in the few Steinernematidae which are less host specific, as experimental results reveal. Also a few facultatively parasitic Diplogasteridae and Rhabditidae appear promising. In the biological control of insect pests, parasitic nematodes as yet play a relatively small part.

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DISKUSSION

P. A. van der Laan: In Amsterdam wird jetzt mit einer Kultur der Steinernematide von Dr. Dutky (USDA) gearbeitet, die ich von Dr. Oostenbrink bekommen habe. Es gelang, die Raupen von der Wachsmotte und der Kohlraupe zu infizieren mit einer Mikropipette, gefüllt mit einer ganz geringen Anzahl Nematoden, und die Infektion war bis nahe 100 % gelungen. Unter natürlichen Umständen war der Prozentsatz viel geringer. Vielleicht ist eine Massenzucht der Nematoden im Laboratorium auf diese Weise möglich.

M. Oostenbrink: Es wäre noch zu bemerken, dass bei der Bekämpfung von Insekten mit Nematoden man mit der von Dr. Rühm und Dr. van der Laan genannten Art der Steinernematidae vielleicht doch noch die beste Möglichkeit hat. Wenn man Insektenlarven mit diesen Nematoden inoculiert, kann die Zahl der Nematoden innerhalb einer oder zweier Wochen bis auf 150000 pro Larve steigen. Das betrifft Adulte und Larven.

Aus Amerika werden in einem speziellen Bericht praktische Resultate von Verspritzen einer Suspension dieser Nematoden gegen Obstinsekten gemeldet. Bei eigenen Untersuchungen mit denselben aus Amerika erhaltenen Nematoden zeigten sich aber die Umstände wichtig und sind noch schwierig zu beherrschen. Hat Dr. Rühm mit diesen Nematoden Erfahrungen?

Antwort: Mit Steinernematiden habe ich noch keine praktischen Erfahrungen sammeln können.

H. Goffart: Ich kann die Schlussfolgerungen Dr. Rühms auch für *Phyllotreta*-Arten bestätigen. Nach meinen Untersuchungen hängt der Nematodenbefall weitestgehend von der Lebensweise der *Phyllotreta*-Arten während ihres Larvenstadiums ab.

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SOME ASPECTS OF THE BIOLOGY AND ECOLOGY OF STEM EELWORMS

BY

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In 1888 RITZEMA BOS brought a number of nominal species together in the species *Tylenchus devastatrix* (the present *Ditylenchus dipsaci* (Kühn)), but at the same time noticed the fact that stem eelworms from different plants showed different host preferences. Since then the existence of biological races of this eelworm has received continuous attention. Observations by OORTWIJN BOTJES (1913) and cross inoculation experiments by VAN SLOGTEREN (1920), GOODEY (1922), HODSON (1926) and others have shown that these host preferences are constant and that the biological races are well distinguishable biological units. As pointed out by BOVIEN (1955) a number of questions concerning their interrelationships have still to be answered such as the possibility of interbreeding and whether new races can originate in this way.

All the races of *Ditylenchus dipsaci* cause the same symptoms in their hosts: dissolution of the middle lamellae, swelling and distortion. *D. destructor* Thorne, which was formerly included in the same species, causes different symptoms.

Attempts to distinguish the biological races morphologically have failed until now. STEINER's (1956) suggestions have not yet been substantiated. KIRJANOVA's (1951) *D. allii* (Beyerinck) seems to lack morphological characters which distinguish it from the other races. Moreover an objection must be raised against the use of the specific name *allii* Beyerinck. This suggests that the stem eelworm race found in onions in Russia is the same as that occurring on the island Goeree-Overflakkee, in the Netherlands. This is by no means sure, however, as many races may occur in onions.

To distinguish the different races their host ranges must be investigated. Reliable results can only be obtained by inoculation experiments. For these it is necessary in the first place to have a suf-

ficient supply of inoculum. Collection of infested plants in the field cannot be relied upon for such investigations. Usually enough material for a whole range of experiments cannot be found in one locality and stem eelworms collected from the same plant species but in different places should not be mixed as they may belong to different races. A continuous supply of a certain stem eelworm race can only be secured by maintaining a stock culture. Potato tubers, mangolds, teazel and flower bulbs are suitable substrates for culturing large numbers of certain races of the stem eelworm. For other races e.g. the rye race and the red clover race, no host plants have been found yet which, by having tubers or voluminous stems, produce enough tissue to support a large population. Here tissue cultures might help. They might also be a suitable substrate for the eelworms in investigations on problems like the mode of reproduction and the existence of interbreeding between races. With stock cultures, investigations of the host range of a race can be repeated or the susceptibility of new plant species may be tested if comparison with new data on other races is necessary.

In the literature much information on hosts of different races of the stem eelworms can be found. Nevertheless it is often difficult to compare these data, as very often the host ranges investigated by different authors do not overlap sufficiently. If in host range tests a standard host range were included such comparisons would be much easier.

Table I shows that eleven races known from the literature and from the author's experiments can be distinguished by inoculating nine plant species. It would be an advantage if these nine plant species were adopted generally as a standard test range. Further research on new races may indicate that other plants should be added to the standard range.

There has been a tendency, undoubtedly induced by the results of host range tests with *Heterodera* species, to investigate the susceptibility of plant species related to the host from which the stem eelworms were collected. Mostly these plants were not susceptible and the eelworm races investigated were therefore considered highly specialized. However, the hosts of a stem eelworm race do not belong to a limited number of plant families. Where sufficient plant species have been tested the race always appears to be rather polyphagous. Some of the hosts are susceptible to attacks by different races. Thus onions are susceptible to the onion, rye, teazel, daffodil and two potato races and rye to the rye, onion and two potato races.

TABLE I

The distinction of eleven biological races of the stem eelworm on nine plant species.

	Teazel race	Red clover "	White clover "	Lucerne "	Rye "	Onion "	Potato " 1	Potato " 2	Hyacinth "	Daffodil "	Tulip "
Teazel (*Dipsacus fallorum*)	+++	—			—	—	—	—			
Red clover (*Trifolium pratense*)	—[1]	+++	—[3]	—[3]	—	—	—	—		—	—
White clover (*T. repens*)		—[2]	+++		—	—	—	—			
Lucerne (*Medicago sativa*)		—[2]	—[3]	+++	—	—	—	—			
Peas (*Pisum sativum*)	—	++			—	+++	+++	+++			
Potato tubers (*Solanum tuberosum*)		—			+	+	+++	++		—	
Daffodil (*Narcissus* sp.)		—			—	—	—	—	—[4]	+++[4]	+++[5]
Hyacinth (*Hyacinthus orientalis*)		—			—	—	—	—	+++[4]	—[4]	+++[5]
Tulip (*Tulipa Gesneriana*)		—			—	—	—	—	—	—	+++[5]

1) Courtney (1952)
2) Goodey (1922)
3) Frandsen (1951)
4) van Slogteren (1920)
5) Southey (1957)

Knowledge of the host range of the different stem eelworm races indicates which crops can be grown without risk if a certain plant species is found attacked, e.g. red clover after diseased onions, rye or oats. It is of little use however for devising crop rotations intended to reduce the damage in susceptible crops. Table II shows the influence of a number of crops on the degree of infestation. These data were collected in the same way and largely from the same fields mentioned by SEINHORST (1956). Each observation (degree of infestation and multiplication rate) is based on two to six plots of about 7 sq. m within one field.

Apart from rye and onions there is no difference between the influence of hosts and of non hosts on the degree of infestation of the soil. The soil type determines what happens to the eelworms both in summer and in winter (SEINHORST 1956). On heavy soils low degrees of infestation (below 100 stem eelworms per 500 g of soil) generally increase during summer whether a host or non host crop is grown. At degrees of infestation between 10 and 60 stem eelworms per 500 g of soil the increase during summer balances the decrease during winter. On light soils the situation is different. Except when rye, oats or onions are grown, the degree of infestation of the soil mostly decreases during both summer and winter until it is about 5 stem eelworms per 500 g of soil or lower. At this level of infestation the decrease during winter may be balanced by an increase during summer. As damage may occur in many crops at degrees of infestation higher than about 10 stem eelworms per 500 g of soil crop rotation is of no effect on heavy soils. On lighter soils, however, onions can be grown once in three or four years. On the infested sandy soils, rye can be grown from about once in four years to once in two years depending on soil type, whereas in a large area with sandy soils, stem eelworms do not occur although rye is or has been grown there more frequently.

Most probably weeds are very important in maintaining a certain level of infestation. Efficient control of these weeds might therefore reduce the danger of attack by stem eelworms.

The population dynamics of the stem eelworm differ considerably from those of *Heterodera rostochiensis* Woll. for example. In the latter the picture is relatively simple as increase of the population occurs only when one of the few hosts is grown. If no hosts are grown the population decreases. The influence of soil type is not usually very marked. The population density of the stem eelworm, however, fluctuates be-

TABLE II

Rate of increase of stem eelworms on different crops.

Crop	Clay soil with onion race		Sandy soil with rye race	
	Number of stem eelworms per 500 g of soil before crop	ratio number after/before crop	Number of stem eelworms per 500 g of soil before crop	ratio number after/before crop
Oats **)	230	1	712	0,5
Avena sativa	6	3	52	2,3
	1	40	8	0,4
			6	0,5
			3	8
Wheat	225	0,2	22	0,9
Triticum vulgare	18	6	10	0,9
			6	0,7
Barley	25	1	17	1
Hordeum sativum			8	0,4
			8	1
			2	2
			2	2
			0,2	12
Peas *)	17	13	14	0,6
Pisum sativum	14	3,2	13	0,8
	8	10		
Potatoes **)	205	0,2	209	0,2
Solanum tuberosum	62	1	8	1,2
	57	2,4		
	38	1,5		
	22	1,2		
Sugar beet **) and	121	1,1	70	0,9
mangold **)	95	1	46	0,7
Beta vulgaris	66	1,2	28	0,25
	40	1,5	24	0,5
			12	0,04
			8	0,4
			4	0,8
Flax	38	3		
Linum usitatissimum	20	3,4		
Chicory	16	0,3		
Cichorium yntibus	12	4		
Onions **)	7	30		
Allium cepa	degree of infestation after onions (mean of 6 fields) *152* stem eelworms per 500 g of soil			
Rye **)			55	0,7
Secale cereale			31	1,6
			22	5
			21	7
			degree of infestation after rye (mean of 9 fields) *374* stem eelworms per 500 g of soil	

**) hosts of the onion and the rye race.
*) host of the onion race.

tween rather narrow limits. These limits depend on soil type and relatively little on the host plants cultivated, few of which cause a considerable rise in the degree of infestation of the soil. With other polyphagous species of plant parasitic nematodes a similar situation exists. They may be found consistently over large areas but only rarely occur in numbers exceeding a certain limit, which may be different in different places. Their importance as plant pathogens depends largely on whether this upper level exceeds the level at which damage can be expected in some of the crops grown.

ZUSAMMENFASSUNG

Es wird eine kurze Übersicht über die Probleme und die Ergebnisse von Untersuchungen über die biologischen Rassen des Stengelälchens gegeben. Die Notwendigkeit des Anlegens von Vorratszuchten von Stengelälchen jeder Herkunft und der Untersuchung der Empfindlichkeit einer Standardreihe von Wirtspflanzen für eine bessere Vergleichsmöglichkeit der verschiedenen Untersuchungen wird betont.

Im allgemeinen ist nicht die angebaute Pflanzenart, sondern der Boden bestimmend für Grösse und Richtung der Änderungen im Verseuchungsgrad. Auf schweren Böden ist bei 10-60 Stengelälchen je 500 g Boden die Zunahme im Sommer ungefähr gleich der Abnahme im Winter. Auf leichten Böden schwankt der Verseuchungsgrad meist zwischen 0 und 10 Stengelälchen je 500 g Boden. Nur durch Anbau von Roggen, Zwiebeln und bisweilen Hafer kann er weit über diese Zahl hinausgehen.

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DISCUSSION

F. G. W. JONES: Although I would agree that *Heterodera* species behave differently from *Ditylenchus dipsaci,* there are certain soils in which *Heterodera schachtii* does not appear to increase. Also when host plants are grown, increases are not always obtained, even though the soil is favorable.

H. GOFFART: Wie erklärt sich die Vermehrung der Stengelälchen an Nichtwirtspflanzen, die sich aus der Tabelle ergibt.

ANTWORT: Die Älchen vermehrten sich sehr wahrscheinlich an Unkräuter.

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STEM EELWORM INVASION OF SEEDLING SUGAR BEET AND DEVELOPMENT OF CROWN CANKER

BY

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Stem eelworm, *Ditylenchus dipsaci* (Kühn) Filipjev, has been known to attack beet and mangolds on the Continent of Europe for at least 50 years, causing crown canker. HIJNER & DEN OUDEN (1953) recorded losses of up to 30 % in mangolds in the Netherlands and considered that the trouble was increasing slowly, whilst the most serious damage to beet by this eelworm has been reported from Switzerland by SAVARY (1954). RIETBERG (1953) gave the position regarding beet stem eelworm in many countries, as known at that time.

In England, GOODEY (1929) was the first to record stem eelworm attacking mangolds and, later, ROEBUCK & HULL (1937) described its occurrence in sugar beet and the symptoms produced.

In all these previous observations, the general conclusion reached was that the crown canker arose as a result of eelworms invading the 'root' at about soil level in the late summer and autumn. Canker was usually more extensive in mangolds than in sugar beet. JOHNSON (1937) attempted to infest mangold seedlings artificially but found that, though the seedlings became distorted, the eelworm infestation soon died out and the plants grew normally.

In early June 1953, symptoms of stem eelworm attack were first noticed on sugar beet in the field in various parts of England. In cases where the growing point was invaded, blindness of the plants resulted, with subsequent stunting and multiple-crown development; more commonly only leaf and petiole distortion and bloating was apparent. Crown canker developed in the late summer and autumn, though less commonly on the multiple-crown plants.

Since damage of economic importance was clearly being caused in the 1953 attacks (DUNNING, 1954a), further detailed investigations

1) Work done whilst with Sugar Beet Pests Section, School of Agriculture, Cambridge.

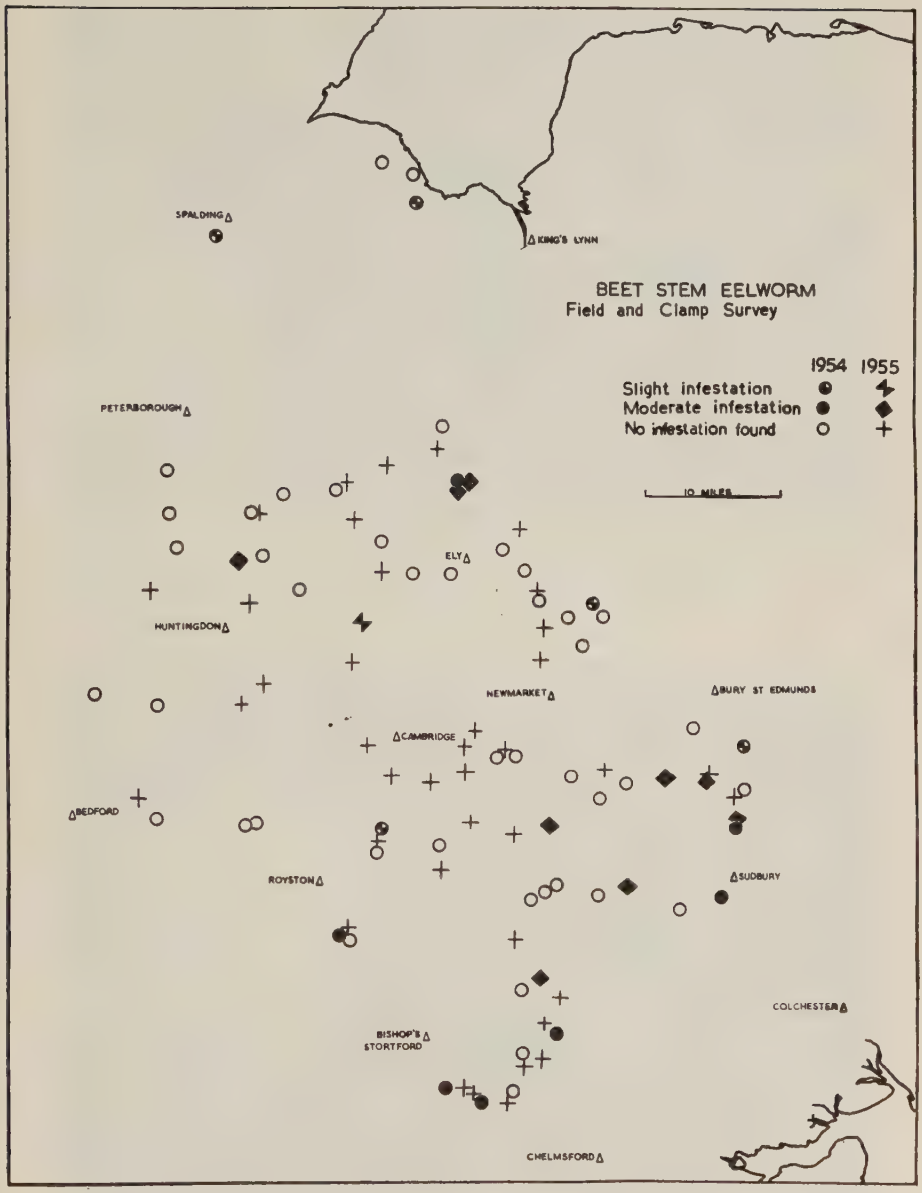

Fig. 1. Distribution of beet stem eelworm infestation in Cambridge area, as found by field and clamp survey (1954 — present at 22 % of sites; 1955 — present at 21 % of sites).

were made during 1954 and 1955, some results of which have already been published (Dunning, 1954b, 1956).

The race of *D. dipsaci* attacking sugar beet and mangolds is the general field race. *D. destructor* Thorne has not been found attacking beet or mangolds in England but, when beet was inoculated artificially, crown canker similar to that caused by *D. dipsaci* was produced. Dallimore & Thorne (1951) have described the symptoms of *D. destructor* attack on beet in the U.S.A.

Distribution of infestation in England.

In 1954 and 1955 during Nov.-Dec., a survey of fields and roadside clamps was made within a 30 mile radius of Cambridge. A total of 103 sites were examined and, although no serious attacks were found, stem eelworm canker was present in slight or moderate amounts at 22 % of the sites in 1954 and 21 % of the sites in 1955 (Fig. 1). Since the summers of 1954 and 1955 were so different, it was most interesting to find the prevalence of canker the same in these two years.

From the results of this survey, and also more general observations, it is clear that this race of stem eelworm is virtually endemic in England. Attacks are found on all soils, though mainly on the heavier types. As beet is not a particularly good host, extensive seedling invasion and consequent damage occurs mainly under cool moist conditions when growth is slow (e.g., Spring, 1953).

Seedling symptoms.

These have been described and illustrated in previous publications by the present author. In conclusion, with the further observations made, they may be summarized as follows (Fig. 2). The normal symptoms are bloating and malformation of the petioles and mid ribs of the cotyledons, and rough leaves up to about the sixth pair. These symptoms persist until the death of the leaf. Blindness, as a result of invasion of the actual growing point, is less common, but by no means abnormal, occurring in the field to a variable extent each year and being readily produced experimentally. It is almost invariably associated with bloat of the cotyledons or leaf petioles. Blind plants remain severely stunted, but axillary buds develop, leading to a multi-crowned root (Fig. 3).

Young seedlings that had grown in infested soil were stained at various stages and it was found that invasion of the hypocotyl or petio-

Fig. 2. Stem eelworm symptoms on seedling-beet. Left hand plant — bloating and malformation of first pair of rough leaf petioles with death of growing point. Centre and right hand plants — bloating of cotyledon petiole only.

Fig. 3. The end result of seedling blindness caused by stem eelworm invasion in mangold. Comparison of normal healthy root and the stunted root with multiple crown plus canker in October (Canker not readily discernible, being internal from the central fissure).

4

5

Fig. 4. Early stages of canker in leaf scar region of beet in September. Note raised, pustular nature.

Fig. 5. Mangold showing site of inwardly progressing canker at leaf scar (marked by arrow), approximately 7 cms. above soil level. October.

Fig. 6. Mangold (left) and beet (right) taken from field in November and cross sectioned (at approximate level of arrow in Fig. 5) to show main sites of canker. These have developed from early infestations in the leaf petioles of two pairs of rough leaves.

les by one or more eelworms can occur immediately the shoot emerges from the fruit.

Development of canker.

The previous suggestion (DUNNING, 1956) that there must be some connection between the spring invasion and the development of crown canker in the late summer and autumn has now been confirmed by further observations.

Seedlings showing petiole distortion in May were observed throughout the summer and autumn. After the death of the infected petiole in late June there was no further evidence of infestation until late August, from which time inwardly penetrating canker usually became increasingly obvious at the scar of the infested petiole (Fig. 4 & 5). In the case of multiple-crown plants, canker sometimes started at the site of the original dead growing point (as in Fig. 3), but often was much less evident than in those roots which, as seedlings, had simply shown the more normal petiole bloating. (See DUNNING 1954a for numerical data.) It must be assumed that the death of the growing point in the seedling, causing serious stunting of the plant and axillary bud development, produces less favourable conditions for survival and multiplication of the eelworms than in the normal, rapidly growing plant.

In Fig. 6, a horizontal section of a mangold and a beet at the level of the lower leaf scars illustrates canker developing in the four quarters as a consequence of early infestations of opposite pairs of petioles. Such separation of invasion sites is soon masked as canker and secondary rots progress.

In addition to this development of crown canker from petiole infestation it seemed probable that simple invasion of the crown from the soil during the summer and early autumn was possible, and an experiment was made to determine the relative importance of these two methods of crown invasion.

Plots of known infested and uninfested (but not sterilized) soil were used. All seed was sown on April 7, some in plots of infested soil, but the majority in pots of infested or sterilized soil. During the last week of May seedlings growing in the plots of infested soil were thinned out, leaving a mixture of seedlings with and without symptoms of infestation. Infested seedlings were those showing bloating on one or more petioles: blind seedlings were discarded.

At the same time, seedlings from the pots of sterile soil were trans-

planted into the plots of infested or uninfested soil, whilst the seedlings from the pots of infested soil were first separated into those showing

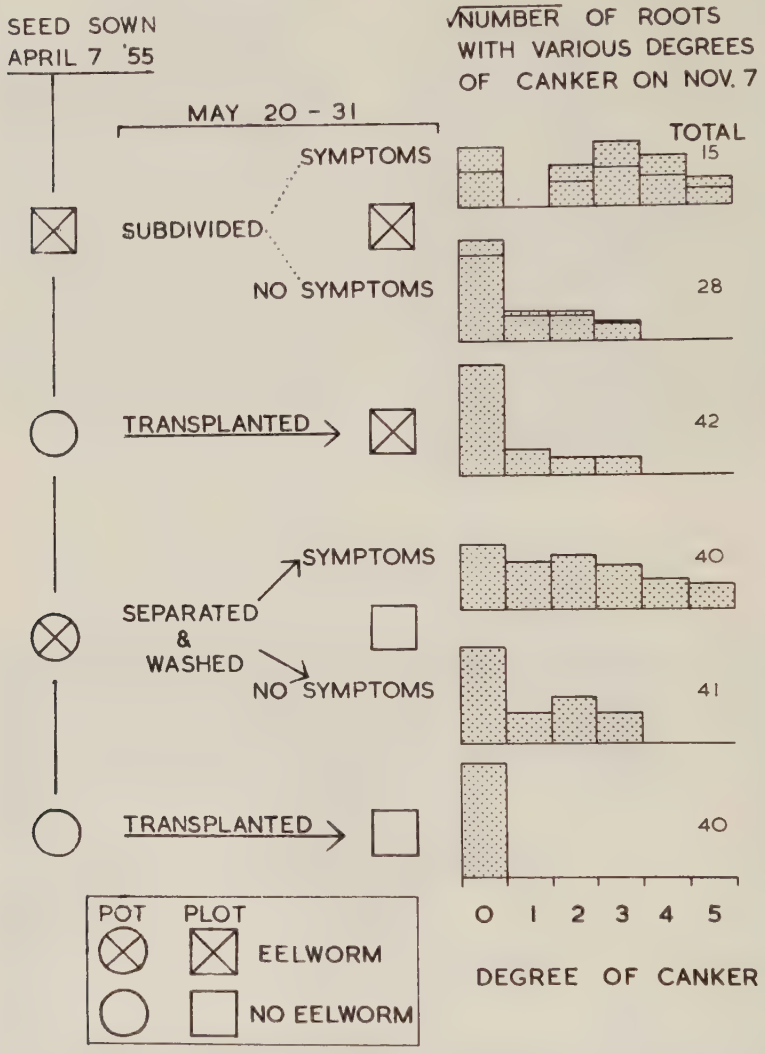

Fig. 7. Seedling treatments given and extent of autumn crown canker resulting in a beet and mangold infection experiment. See text for full details (Beet and mangold combined).

symptoms and those showing no symptoms, then very thoroughly washed in running water to remove all external eelworms and a sample of each group transplanted into an uninfested plot. All these various

treatments are shown diagrammatically in Fig. 7. There were four sowing treatments, two being subdivided at the end of May, making six final treatments.

On Nov. 7 the roots in the six treatments were lifted, washed and classified according to the degree of canker as follows: 0 - healthy; 1 - very slight superficial canker; 2 - slight canker; 3 - moderate canker, up to ⅛ of crown; 4 - severe canker, up to ½ of crown; 5 - very severe canker, more than ½ of crown. Results obtained are shown in Fig. 7, square root transformations having been used. The final number of roots classified per treatment ranged from 15 to 42 as shown in Fig. 7, and, in the first two treatments, with only 15 and 28 roots, the numbers are also graphed after 'scaling-up' to 40 roots for ease of comparison.

The results from beet and mangold, which were grown in equal numbers, have been combined, it being sufficient to note that the mangolds showed somewhat more severe canker on November 7 than did the beet (cf. Fig. 6). This is a difference frequently observed in the field, and one noted by previous authors, and is probably due to both greater susceptibility to seedling infestation and greater susceptibility to the development of canker.

Though numbers of plants per treatment were small it is possible to draw the following conclusions:

(1) Healthy seedlings transplanted into stem eelworm infested soil can become infested to a limited extent during the summer, slight to moderate crown canker developing.

(2) Crown canker is much more likely to occur and to be more serious in plants that originally showed infestation in May in the form of bloating, galling and malformation, irrespective of whether the plants grew during the summer and autumn in infested or uninfested soil.

(3) Seedlings can be infested without showing obvious symptoms in May, and crown canker may develop from these latent infestations by November, even when the roots are grown throughout the summer and autumn in uninfested soil.

ZUSAMMENFASSUNG

Es wird ein Überblick über die Literatur des Stengelälchenbefalls an Rüben gegeben. Beobachtungen über die Verteilung in England führten zu dem Ergebnis, dass der Nematode in diesem Lande endemisch ist. Die Keimlingssymptome können in eine normale Schwellung der Blattstiele und in eine weniger häufige Zerstö-

rung des Vegetationspunktes unterteilt werden. Aus Beobachtungen und Versuchen wird geschlossen, dass die im Herbst auftretende ernste krebsartige Kopfrissigkeit sehr wahrscheinlich aus einem frühen Befall der Blattstiele entsteht, besonders dort, wo die Schwellung deutlich ist, während die weniger schwere Rissigkeit auf einen Älchenbefall des Rübenkopfes während des Sommers und Herbstes zurückzuführen ist.

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DISCUSSION

M. Oostenbrink: Does seed play a role in the distribution of the stem eelworm in beets?

R. A. Dunning: The eelworm can be seedborne, but this appears to be of little practical importance in England*, though it may well be in other countries. (* See Dunning, 1956.)

J. W. Seinhorst: Did you ever see symptoms in leaves and petioles at the time the eelworm cankers had started to develop?

R. A. Dunning: No, although some evidence was obtained in the case of bolters that eelworms were present without producing obvious symptoms.

J. A. Hijner: You show on the map (Fig. 1) the distribution of stem eelworm in England. Are some spots of heavier infestation due to a short beet rotation or to other susceptible crops?

R. A. Dunning: Some of the sites are associated with frequent growing of onions, but there is little correlation with frequency of beet growing.

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LUCERNE STEM EELWORM IN GREAT BRITAIN

BY

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Lucerne stem eelworm (*Ditylenchus dipsaci* (Kühn) Filipjev) has long been known as an important pest in most of the lucerne-growing areas of the world and is particularly troublesome in areas of high rainfall and in irrigated districts. In this lecture I hope to say something of its occurrence and of efforts made to combat it in Great Britain, with particular reference to the Eastern Counties where nearly 50 % of the country's lucerne acreage is grown.

This pest was first found in Great Britain in 1948 (Brown 1953). During the following five years a further six infested fields were discovered all sown wholly or in part with seed imported from Hungary. Since then, coinciding with the increasing preference for the very good Northern French varieties of lucerne (Flamande type and du Puits), there has been a rapid increase in the number of infested fields until at the moment well over 100 are known. There is little doubt that the eelworm occurs wherever these varieties are grown. A survey carried out in the Eastern Counties in 1956 showed that the eelworm infestations were mainly confined to these varieties and that there was extremely little in Provence lucerne which had previously been extensively grown in Great Britain. Of a total of 39 fields of Flamande and du Puits, 21 were infested but only seven of a total of 61 fields of Provence lucerne showed any signs of eelworm attack and there was fairly good evidence that the eelworm had been introduced to most of these fields after establishment of the crop. Similar results have been obtained in surveys in other parts of the country.

Infested plants are stunted and in the early spring often remain flush with the soil surface and may be difficult at first glance to recognise as lucerne plants. They exhibit swollen shoots the leaflets of which are distorted and usually remain folded. Many of these shoots decay but some partially recover and may be recognised by their swollen stem bases. Later in the season, after the first cut, the rapidly-growing new

shoots carry eelworms up with them and swellings may then be found high up the stems.

Symptoms are not visible in the crop until the spring of the year following drilling and the best time to examine fields is when the new growth is about 10 cms high. At this time, circular patches rarely more than 1 metre in diameter may be found although after a very wet winter some of these patches may lose their circular shape particularly on sloping ground. By the second year they have become irregular in shape, averaging 5-6 metres in diameter, and surrounded by a number of small circular patches. Elongation of the patches occurs in the direction of cutting and also on slopes where long narrow strips sometimes 50-60 metres in length may be formed by the eelworms being washed downwards in surface drainage water during the winter. Sometimes patches of 20 metres diameter may occur in these two-year old fields but such patches have probably been formed by the coalescence of several smaller ones. By the third year the infestation has usually spread over the whole field and the eelworm is by then exerting a serious effect on the crop.

Lucerne being a perennial is difficult to kill and, as some shoots are destroyed by the eelworm, further ones are produced by the plant and may be attacked in their turn. Recovery of the crop may be extremely good especially in dry periods unsuitable for eelworm activity. However, the ultimate effect of the eelworm is to thin out and weaken the stand to such an extent that it has to be ploughed up.

Frederiksen (1950) in Denmark showed that in addition to lucerne, sweet clover (*Melilotus alba* Desr) is also a host of this race of stem eelworm. In Great Britain J. B. Goodey and Brown (1956) found that alsike clover could also be severely damaged. These are the only known crop hosts but I have recently found eelworm infestation in two weeds growing in lucerne stem eelworm patches. These were hedge parsley (*Anthriscus sylvestris* (L.) Berth.) and plantain (*Plantago major* L.). The plant of hedge parsley was severely attacked, with swollen stems and rotting at the base of the plant. However in this plant there was a mixed population of *D. dipsaci* and *Aphelenchoides ritzema-bosi* (Schwartz) Steiner. The latter species occurred with the *D. dipsaci* in the swollen tissues and in cortical lesions on the stems and also by itself in the leaves. As this was the only *Anthriscus* plant infested among quite a large number examined it is possible that the activity of the *Aphelenchoides* may have created suitable conditions for the entry and multiplication of the *D. dipsaci*. Lesions on the mid-

veins of the leaves of *Plantago major* have been seen quite commonly in lucerne stem eelworm patches. These have contained specimens of *D. dipsaci* and occasionally a few eggs. It cannot therefore be considered a very efficient host and one wonders if it would be invaded under more normal conditions.

Following the suggestion made by Lopez Cristobal (1946) in the Argentine an attempt has been made to eradicate the eelworm from small circular (1 metre diameter) patches by the use of fire. Waste engine oil was first of all sprinkled liberally on to the patch and the area surrounding it and then burnt. This was followed by the use of a flame gun to scorch the surface layers of the soil and to burn any lucerne residues. This was carried out early in the spring as soon as it was possible to distinguish the eelworm patches. The results of this treatment were extremely disappointing although it is doubtful if it could have been a practical proposition. In some patches there had been some control but in others the eelworm infestation had spread as much as in the untreated patches.

Another control method to which attention has been given is seed fumigation with methyl bromide. This presupposes that the nematode is seed-borne and I shall have more to say later about the reasons for thinking that this eelworm is non-indigenous in Great Britain. The figures I shall quote are concentration time products (C.T.P.) and are given in oz hours/1000 cu ft which is approximately equivalent to milligram hours/litre. T. Goodey (1945) showed that *D. dipsaci* in onion seed could be successfully controlled using a C.T.P. of 600. More recently J. B. Goodey (1949) found that red clover and teazle seed could also be successfully treated using the same C.T.P. figure and that the seed germination was improved. In the spring of 1956 Dr. A. B. P. Page and his co-workers at Imperial College, London supervised the fumigation of 6 tons of lucerne seed. This was done under tarpaulins using a C.T.P. of 1250 and gas samples taken by Dr. Page during the fumigation enabled a satisfactory concentration to be maintained. All the fields drilled with this seed were inspected in the spring of 1957 and eelworm patches were found in most of them. In order to get comparative figures the number of patches found on each field during a 15 minute walk was recorded and it is estimated that about 5 acres of ground were inspected in this period. Table I gives the results of this work for the two varieties du Puits and Flamande W268. There is some evidence of two groups of infestations in the W268 figures and possibly also in the du Puits.

Table I

No. of patches found in 15 minute walk on each field.

W268	17, 0, 12, 18, 1, 3, 3, 16, 30 20, 17.	Total 11 fields
du Puits	1, 18, 18, 7, 14, 9, 18, 10.	Total 8 fields

This result was most disappointing and I began to wonder if my ideas about seed infestation were correct. Fortunately on one of these fields a replicated fumigation trial using the same seed was in progress. The seed had again been fumigated by Dr. Page, this time in airtight fumigation chambers using methyl bromide at three C.T.P. rates: 0, 1250 and 2500. In this trial there were six replicates and the size of each plot was 50 yards × 3½ yards. Table II shows the number of eelworm patches found in each treatment for each of the two varieties.

Table II

Number of eelworm patches on trial plots

	W268	du Puits
Controls	14	2
CTP 1250	2	0
CTP 2500	1	0

The eelworm patches were therefore almost confined to the untreated controls. It was impossible to decide if the patches in the treated plots originated from eelworms which survived fumigation or if the infestation had spread to these plots after establishment of the crop. There was opportunity for this to occur as not only were the plots cut the previous autumn for yield but also the large machines drove across them while cutting the rest of the field.

It is interesting to compare the most heavily infested W268 field (Table I) with the W268 controls in the fumigation trial (Table II). In this field there were 30 eelworm patches per 15 minute examination, giving an estimated six patches per acre. In the trial there were 14 patches in the 6 plots or 65 patches per acre. This is a very rough comparison but it gives some indication of the degree of control achieved by the tarpaulin fumigation.

I intend now to enumerate the other reasons for thinking that this eelworm is non-indigenous and that the infestations have all been introduced with the seed.

1. Few if any of the infested fields have previously grown lucerne or any other known host, certainly not for many years.
2. Stem eelworm attacks on other crops are rare on the light sandy soils

where most of the lucerne is grown. Healthy broad red clover plants can nearly always be found growing in lucerne stem eelworm patches.

3. It was unknown in Great Britain before 1948 and its appearance coincided first with the use of Hungarian seed for a short period of years and secondly with the introduction of the Flamande varieties from France.
4. In May 1957 I visited the French lucerne seed producing areas through the courtesy of the French Ministry of Agriculture and M. RITTER in particular. There was a very close correlation between the incidence of stem eelworm on the two types Provence and Flamande in France and Great Britain.
5. By the spring of the year following drilling, eelworm patches in lucerne crops are always small and very rarely more than one metre in diameter such as would be expected from seed-borne infestation. This may be compared with clover stem eelworm which is a common pest in Great Britain. With this race very large patches and even complete failures are frequently encountered in one year old crops and such attacks almost certainly originate from well-established soil populations.
6. The examination of the W268 unfumigated seed from the fumigation trial revealed the presence of eight larvae of *D. dipsaci* in 2 lbs (about 900 gms) of seed. This seemed a very small number in view of the number of patches in the plots sown with this seed. However this sample was examined about a year after the plots were drilled and the nematodes extracted were all pre-adult (4th stage) larvae. It is possible that other stages of the eelworm may have survived from harvest until drilling the following spring and helped to establish the infestigation. Two other infested samples have been encountered, one containing 14 larvae of *D. dipsaci* in 2 lbs of seed and the other 6 larvae in 14 lbs. Saprophytic nematodes are often extremely numerous in lucerne seed samples.
7. Lucerne seed is not normally produced in Great Britain but this year a small area in an infested field has been left seed for observation purposes. In this plot swellings caused by *D. dipsaci* have been found high up on the stems of the plants, often resulting in atrophy of the flower buds. It is unavoidable that when such infested material goes through the threshing machine eelworm-infested debris will get mixed with the seed and it will be impossible to remove all of this in subsequent cleaning. In some seed heads small numbers of eelworms have been found in the peduncles attaching the seed pods to

the main flower stems and also a few in the pods themselves. It therefore seems certain that the majority of the eelworms are in the debris but it is possible that a small percentage of the seeds may have eelworms attached to them although this has yet to be proved.

In conclusion I should like to consider briefly why the methyl bromide fumigation under tarpaulins did not give a 100 % control. Dr. Page considers that a satisfactory concentration of gas giving a C.T.P. of 1250 was obtained by this method. It is possible that the seed was too dry, and there may be a lower limit for moisture content below which the pre-adult larvae are resistant. We hope that further work will provide the answer and, eventually, prevent further introductions of the pest.

ZUSAMMENFASSUNG

Schädigung der Luzerne durch Stengelälchen trat in Grossbritannien erstmalig 1948 auf. Während der nächsten 5 Jahre wurden 6 weitere befallene Felder, die mit ungarischer Saat bestellt worden waren, entdeckt. Von 1954 bis 1957 wurden fast 100 infizierte Felder gefunden; die Mehrzahl von ihnen war mit französischen Sorten des Flamand-Typs bestellt worden.

Befallene Pflanzen sind gestaucht und haben angeschwollene Triebe. Viele von ihnen werden vernichtet, einige erholen sich teilweise und können an ihrer angeschwollenen Basis erkannt werden. In der vorgerückten Jahreszeit können die Anschwellungen bis an den oberen Stengelteilen der Pflanzen gefunden werden.

Im Frühling nach dem Jahr der Aussaat sind die Flecken meist rund und selten mehr als 1 m im Durchmesser. Ein Jahr später erreichen sie einen Durchmesser von 5-6 m und haben ihre runde Form verloren. Nematoden können mit dem Oberflächenwasser verschleppt werden und rufen dann lange, schmale Flecke von zuweilen 50 bis 60 m Länge hervor. Wegen der Mehrjährigkeit der Luzerne ist es schwierig, den Befall auszumerzen. Eine Erholung der Befallsstellen tritt besonders in Trockenperioden ein, jedoch bleibt der Bestand dünn und schwach, sodass er umzupflügen ist.

Die Verbreitung tritt im Felde während der Erntearbeiten auf. Hierbei besteht auch die Gefahr einer Infektion anderer Luzernefelder. Schwedenklee wurde in einem Versuch auf einem infizierten Feld stark geschädigt. Gesunde Rotkleepflanzen können öfters im Zentrum von befallenen Luzerneflecken gefunden werden. Zwei Unkräuter (*Anthriscus* und *Plantago*) werden vom Nematoden befallen, sind aber gewiss keine bedeutenden Wirtspflanzen.

Allem Anschein nach ist der Nematode saatgebunden. Die Stärke der Befalls variiert nach Sorte und innerhalb der Stöcke der gleichen Sorte. Kleine Mengen von Stengelälchen wurden in verschiedenen Proben von Luzernesaat gefunden. Die geringe Grösse der ersten Befallsstellen und die Tatsache, dass einige Felder schon früher stark mit Luzerne bestellt worden waren, stützt die Ansicht, dass der Nematode nicht endemisch ist. In einem Wiederholungsversuch, der angelegt war, um die Wirkung einer Entseuchung mit Methylbromid zu untersuchen, waren die Nematodenflecken hauptsächlich auf die unbehandelten Stellen begrenzt.

Ein Besuch der französischen Luzernesaat-Vermehrungsflächen fand im Mai 1957 statt. Stengelälchen waren auf allen Flächen anwesend, aber hauptsächlich

in dem wichtigen Gebiet südlich von Paris, wo 40 % der Felder befallen waren. Sehr geringer Befall wurde in der Provence gefunden.

Versuche zur Bekämpfung der Nematoden im Feld während des Frühjahrs durch Flammenwerfer erwiesen sich als erfolglos. Es besteht Hoffnung, die weitere Einschleppung von Älchen durch Behandlung der Saat mit Methylbromid zu verhindern.

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DISCUSSION

J. D. Bijloo: Refering to the influence of the moisture content of the seed on the results of fumigation, the cyst contents of *Heterodera rostochiensis* Wr. are not killed so easily with methyl bromide when completely dry as when they are damp.

E. B. Brown: It is possible that not only must the seed be below 15 % moisture content to prevent injury but that there is also a lower limit perhaps 11 or 12 % or even higher below which *D. dipsaci* pre-adult larvae are resistant to treatment.

Mary T. Franklin: Since *Aphelenchoides ritzema-bosi* normally enters its host (e.g. chrysanthemum) through the stomata, in the case of *Anthriscus* where both this nematode and *D. dipsaci* were present, could the presence of *Aphelenchoides* have been the cause of entry of the *D. dipsaci*?

E. B. Brown: I have found *A. ritzema-bosi* in cortical stem lesions on both lucerne and *Anthriscus* and I think I am correct in saying that stomata do occur on the stems of these plants. As I have examined many other *Anthriscus* plants in lucerne stem eelworm patches without finding stem eelworm infestation in them I put forward the possible explanation that the lesions made by the *Aphelenchoides* may have enabled the *D. dipsaci* to enter and that the activity of this species created suitable conditions for the *D. dipsaci* to multiply. I have previously found these two species living together in the stems of pyrethrum (*Chrysanthemum cinerariaefolium* (Trev.) Vis.)

M. Oostenbrink: Lucerne stem eelworm seems to be spreading. The situation in the Netherlands is about the same as in England. Until two years ago this nematode was not important. Now quite a number of fields are infested. There are indications that the infestations have been seed-borne and the patches seem to spread quickly.

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DITYLENCHUS DIPSACI AN ZWIEBELN IN MITTELDEUTSCHLAND

VON

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Der intensive Zwiebelanbau in verschiedenen Gebieten Mitteldeutschlands hat verständlicherweise ein verstärktes Auftreten bodenständiger Krankheiten und Schädlinge dieser Kulturpflanze zur Folge. Fast jährlich spielen der falsche Mehltau der Zwiebel (*Peronospora schleideni* Ung.) und die Zwiebelfliege (*Phorbia antiqua* Meigen) eine Rolle und in gewissen Jahren gewinnt der Zwiebelbrand (*Tuburcinia cepulae* Liro) Bedeutung. Allgemein bekannt sind auch Schäden durch *Ditylenchus dipsaci* Kühn. In den Meldungen über das Auftreten von Krankheiten und Schädlingen der Kulturpflanzen im „Nachrichtenblatt für den Deutschen Pflanzenschutzdienst" ist letztgenannter Schädling bisher zwar niemals erwähnt worden, aber wie Nachforschungen in den Zwiebelanbaugebieten Mitteldeutschlands ergaben, ist der an Zwiebeln schädlich werdende Stamm dieses Nematoden weit verbreitet und wiederholt schädlich geworden, nach den Beobachtungen der Praxis jedoch trotz enger Fruchtfolge nicht in jedem Anbaujahr auf gleicher Fläche. Zur Erklärung wurden Temperatur- und Niederschlagsverhältnisse der aus eigener Anschauung bekanntgewordenen Schadjahre 1949 und 1956 sowie der Jahre ohne Schadauftreten 1955 und 1957 verglichen. Dabei konnten in der Literatur (z.B. Filipjev und Schuurmans Stekhoven 1941, Goffart 1949, 1951, Seinhorst 1950, 1956, u.a.) bekannte Tatsachen bestätigt und unsere Kenntnisse über diesen Nematoden erweitert werden.

Die eigenen Beobachtungen beziehen sich auf Schadauftreten mit Totalausfällen im Jahr 1949 im Raum von Erfurt und im Jahr 1956 in den Gebieten um Aschersleben, Quedlinburg, Sondershausen (nördlich des Kyffhäusers) und Dresden. Aus dem gleichen Jahr wird übrigens von Böhm (1956) ein Massenauftreten aus dem Raum um Wien gemeldet. Bei den eigenen Feststellungen im Jahr 1949 und im Jahr 1956 in den Gebieten von Erfurt, Aschersleben, Quedlinburg und Sondershausen handelte es sich um Schädigungen von Saatzwiebeln, in Dresden 1956 dagegen um Befall von Schalotten.

Von besonderem Interesse sind dabei Beobachtungen auf einer Versuchsfläche unseres Instituts, auf der wir im Jahr 1956 Totalschaden feststellen mussten. Auf der gleichen Fläche hatte ich im Jahr zuvor Zwiebeln für Versuche zur Bekämpfung der Zwiebelfliege angebaut. Auf den mit Erfolg gegen die Zwiebelfliege behandelten Parzellen standen in diesem Jahr die Pflanzen einwandfrei, auf den Kontrollparzellen waren nur die durch die Zwiebelfliege verursachten Fehlstellen zu verzeichnen, auf keiner Parzelle fielen uns Pflanzen auf, die die für *D. dipsaci* typischen Schädigungen zeigten.

Zur Prüfung einer eventuellen Nachwirkung der Behandlung gegen die Zwiebelfliege wurden im Jahr 1956 auf der gleichen Fläche erneut Zwiebeln angesät. Durch *D. dipsaci* wurden im genannten Jahr die meisten Pflanzen bereits im Keimpflanzenstadium vernichtet, die zunächst durchgekommenen Pflanzen zeigten später die für den Nematodenbefall typischen Verkrümmungen.

Zur weiteren Nachprüfung haben wir im Jahr 1957 auf der gleichen Fläche noch einmal Zwiebeln und darüber hinaus weitere Kulturpflanzen, die als Wirtspflanzen für *D. dipsaci* bekannt sind, in dreifacher Wiederholung auf 2 m² Parzellen angebaut. Trotz noch vorhandener Bodenverseuchung mit *D. dipsaci* konnten wir im Jahr 1957 jedoch keinen Befall der Zwiebeln feststellen. Im Juli führten wir eine Kontrolle der angebauten Kulturpflanzen und der auf den Parzellen gefundenen Unkräuter durch. Wir prüften gleichzeitig die Unkrautflora auf einer weiteren Fläche in der Flur von Aschersleben, die im Jahre 1956 ebenfalls starke Schäden durch *D. dipsaci* gezeigt hatte und im Jahr 1957 mit Weizen bestellt war. Diese Untersuchung hatte das in der Tabelle I wiedergegebene Ergebnis.

Die Vogelmiere (*Stellaria media* Dillenius) war auch 1956 stark befallen gewesen. Neben dieser waren uns im Sommer 1956 Knötericharten, insbesondere *Polygonum aviculare* L. und *P. lapathifolium* L. durch starken Besatz aufgefallen, für die wir jedoch 1957 nur schwachen Befall feststellen konnten.

Aus der Tabelle I soll nicht der Schluss gezogen werden, dass es sich bei den in den verschiedensten Pflanzen festgestellten Ditylenchen nur um einen für die Zwiebeln gefährlichen Stamm gehandelt hat. Für derartige Feststellungen ist die Untersuchungszeit noch zu kurz.

Das bisher Gesagte zeigt: starke Schädigung 1949 im Raum von Erfurt, starke Schädigung 1956 im Raum von Aschersleben und an anderen Orten Mitteldeutschlands, dagegen 1955 und 1957 auf der 1956 stark geschädigten Fläche bei Aschersleben kein Befall.

Tabelle I

Im Sommer 1957 von Ditylenchus dipsaci befallene Pflanzen.

(Nachbau auf Zwiebelfläche mit Totalschaden im Sommer 1956).

	Befallsstärke *)
I. Kulturpflanzen:	
Pisum sativum L.	++
Fagopyrum esculentum Moench	++
II. Unkräuter:	
Panicum crus galli L.	++
Agropyrum repens P. B.	++
Poa annua L.	+
Polygonum aviculare L.	+
P. persicaria L.	+
P. convolvulus L.	+
Chenopodium polyspermum L.	+
Stellaria media Dillenius	+++
Melandrium noctiflorum Fries	+
Convolvulus arvensis L.	+
Anagallis arvensis L.	+
Mentha arvensis L.	+
Solanum nigrum L.	++
Veronica agrestis L.	+
Plantago major L.	+
Sonchus oleraceus L.	+
S. asper Allioni	+
Senecio vulgaris L.	+
Cirsium arvense Scopoli	++

*) + = schwach
++ = mittel
+++ = stark

Vergleichen wir dazu die Witterungsverhältnisse in den beiden Befallsjahren 1949 und 1956 für die Gebiete Erfurt und Aschersleben. Für die Temperatur ergeben sich keinerlei Übereinstimmungen (Tab. II). Im Jahr 1949 war es in Erfurt im April zu kalt, der Mai entsprach etwa dem langjährigen, in diesem Fall 100-jährigen Mittel, der Juni war zu warm. In Aschersleben war 1956 demgegenüber der April um

Tabelle II

Langjährige Mittel und Monatsmittel der Lufttemperatur in Erfurt 1949 und Aschersleben 1956 für die Monate April bis Juni.

	Erfurt		Aschersleben	
	100-jähr. Mittel	1949	40-jähr. Mittel	1956
April	10,9	7,8	5,2	7,9
Mai	12,7	12,4	13,3	13,1
Juni	13,9	15,7	13,8	15,9

2,7° zu warm, der Mai entsprach etwa dem 40-jährigen Mittel, der Juni war ebenfalls zu warm.

Die Niederschläge (Tab. III) weichen im April voneinander ab.

Tabelle III

Prozent Niederschläge vom 40-jährigen Mittel für die Monate April bis Juni in Erfurt 1949, Aschersleben 1955 bis 1957 und Dresden 1956.

	Erfurt	Aschersleben			Dresden
	1949	1955	1956	1957	1956
April	88	100	140	53	176
Mai	133	93	223	49	66
Juni	101	273	85	62	218

Während in Erfurt nur 88 % vom 40-jährigen Mittel gemessen wurden, lagen sie in Aschersleben 1956 mit 140% über dem Normal. Der Juni entsprach in Erfurt 1949 der Norm, in Aschersleben 1956 war er mit 85 % zu niederschlagsarm. Übereinstimmung ergibt sich aber für den Mai, der in beiden Gebieten in den Beobachtungsjahren über der normalen Niederschlagsmenge, in Aschersleben 1956 sogar beträchtlich höher lag.

Dass in diesen beiden Fällen tatsächlich der Niederschlagsreichtum des Monats Mai den Ausschlag gegeben hat, beweist die Tab. III mit einer Gegenüberstellung der Niederschläge im April, Mai und Juni des Befallsjahres 1956 und der beiden befallsfreien Jahre 1955 und 1957 auf gleicher Fläche in Aschersleben. Die Mai-Niederschläge lagen 1955 wenig und 1957 stark unter normal, im Jahre 1956 dagegen weit darüber.

Es wäre aber falsch, aus diesen Beobachtungen zu schliessen, dass ganz allgemein für *D. dipsaci*-Befall von Zwiebelgewächsen die Niederschläge des Monats Juni ausschlaggebend sind. Ich erwähnte, dass der Nematode 1956 bei Dresden Schalotten stark geschädigt hat. Tab. III zeigt die von April bis Juni 1956 gemessenen Niederschläge für Dresden. Danach war der Monat Mai gerade regenarm, während der April und der Juni weit über dem Normalmass lagen. Ausschlaggebend war hier zweifellos der April.

In den Jahren 1949 und 1956 handelte es sich bei Erfurt und bei Aschersleben um Befall von im Frühjahr gesäten Küchenzwiebeln. In Mitteldeutschland erfolgt die Aussaat auf den gefährdeten schweren Böden zumeist in der ersten Aprildekade. Bei der üblichen Frühjahrswitterung laufen diese etwa Anfang Mai auf. Nach Goffart

(1951) sind die Pflanzen besonders gefährdet, wenn es „zur Zeit der Bildung des zweiten Blattes zu einem Stillstand im Wachstum kommt" und nach SEINHORST (1956) kommt es zu starken Ausfällen, wenn Aktivität und kritisches Stadium der Pflanzen zusammenfallen. Dieser Zustand ist in Mitteldeutschland für die Saatzwiebeln im Mai gegeben. Die Schalotten dagegen wurden bereits im März gesteckt; für sie musste demnach die hohe Feuchtigkeit im April den Ausschlag geben.

Diese Beobachtungen bestätigen, dass der Feuchtigkeit für den Befall der Zwiebelgewächse durch *D. dipsaci* Bedeutung zukommt, und dass die durch die Bodenfeuchtigkeit angeregte Aktivität des Nematoden und das anfällige Entwicklungsstadium der Pflanze zusammenfallen müssen. Die Zwiebeln bleiben trotz Bodenverseuchung befallsfrei, wenn die Feuchtigkeit im Boden zu dieser Zeit nicht ausreicht. Es erklären sich damit die für Mitteldeutschland gegebenen Verhältnisse, dass trotz weiter Verbreitung des Zwiebelstammes von *D. dipsaci* Schäden nicht in jedem Anbaujahr verzeichnet werden. Zur Feststellung über das Ausmass der Verseuchung der mitteldeutschen Zwiebelflächen sind Untersuchungen eingeleitet worden, über die nach Abschluss berichtet werden wird.

In den Jahren ohne Zwiebelanbau und in den Jahren mit für den Zwiebelbefall ungünstigen Bedingungen hält sich der Zwiebelstamm von *D. dipsaci* an anderen Pflanzen, vor allem an Unkräutern. Besondere Bedeutung kommt dabei zweifellos der als Wirtspflanze schon lange bekannten Vogelmiere (*Stellaria media*) zu. Eine Rolle spielen auch die *Polygonum*-Arten. Welche weiteren Pflanzen für die mitteldeutschen Verhältnisse noch Bedeutung gewinnen, lässt sich jedoch heute noch nicht sagen, da die angeführten Beobachtungen deutlich zeigen, dass kurzfristige Anbauversuche keine Auskunft über den Wirtspflanzenkreis geben, wenn das Zusammentreffen von Aktivität des Nematoden und empfindlichem Stadium der Pflanze nicht gewährleistet ist.

SUMMARY

In central Germany *Ditylenchus dipsaci* is not injurious in onion fields every year. Two years, 1949 and 1956, when injury was outstanding were therefore investigated. Temperatures during the two years showed no similarity but in both years the rainfall was above average during the month of May, and this created favourable conditions for attack at a time when the young plants were in a critical stage. Evidently for a serious attack to develope high soil moisture and the susceptible stage of the plant must occur together. In the persistence of the onion race of *Ditylenchus dipsaci*, weeds play a part, especially *Stellaria media* and species of *Polygonum*.

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DISKUSSION

J. H. Schuurmans Stekhoven: fragt, ob es nicht wünschenswert wäre, Unkrautbekämpfungsversuche zu machen, auch mit „Pre-emergence"-Mittel, um zu sehen, ob diese dazu beitragen können, die Nematoden zu bekämpfen, vor allem in bezug auf die Tatsache, dass *Stellaria* und *Polygonum* bisweilen von diesen Nematoden stark befallen sind.

H. W. Nolte: Wir haben solche Versuche vor, zunächst hatten wir das Unkraut absichtlich wachsen lassen, um den Befall der einzelnen Pflanzenarten zu kontrollieren.

J. W. Seinhorst: Waren die Symptome an Erbsen die normalen Verdickungen?

H. W. Nolte: Nein, in diesem Jahr (1957) haben wir aber auch an anderen Pflanzen nur selten Verdickungen gefunden.

Nematologica II, Suppl. (1957) : 382-386 S — E. J. Brill, Leiden

UNTERSUCHUNGEN ÜBER DEN WIRTSPFLANZENKREIS EINER RÜBENRASSE VON *DITYLENCHUS DIPSACI* IN BADEN-WÜRTTEMBERG [1])

VON

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Untersuchungen über den Wirtspflanzenkreis der Rübenrasse von *Ditylenchus dipsaci* (Kühn) Filipjev sind hauptsächlich von BERNARD (1952) in Belgien, sowie von KOTTHOFF (1950) in Westfalen durchgeführt worden. Auch hat DUNNING (1956) in einer seiner Veröffentlichungen einige Wirtspflanzen einer in England vorkommenden Rübenrasse erwähnt, so dass bisher folgende Pflanzen als Wirte bekannt waren:

Kulturpflanzen: Futter- und Zuckerrüben, Roggen, Hafer, Kartoffeln, Mais, Erbsen, Zwiebeln, Bohnen, Mohrrüben, Gurken, Rote Beete, Mangold, Meerrettich, Flachs, Hanf, Buchweizen, Gelbe Bitterlupinen, Süsslupinen, Wundklee, Schwedenklee.

Unkräuter: Vogelmiere (*Stellaria media* Dill.), Ausgebreitete Melde (*Atriplex patulum* L.), Wie aus der Aufzählung hervorgeht, waren alle bisher gemachten Untersuchungen in dieser Richtung meistens auf Kulturpflanzen beschränkt, während die Unkräuter, die für die Vermehrung und Verbreitung eine ebenso wichtige Rolle spielen, kaum berücksichtigt wurden.

UNTERSUCHUNGEN

Befall an Kulturpflanzen und Unkräutern. Die Erforschung des Wirtspflanzenkreises der Rübenrasse von *Ditylenchus dipsaci* in Baden-Württemberg erstreckte sich über 3½ Jahre. Untersucht wurden die im Untersuchungsgebiet hauptsächlich angebauten Kulturpflanzen: Roggen (Winter- und Sommerform), Winterweizen, Hafer, Gerste, Kartoffeln, Rüben, Sonnenblumen, Mais, Rotklee und Luzerne, sowie die auf den verseuchten Äckern vorkommenden Unkrautarten. Zur Versuchsmethodik sei bemerkt, dass einzelne verseuchte Felder in den

[1]) Die vollständige Arbeit wird an anderer Stelle veröffentlicht werden.

Jahren 1954-57 im Frühjahr und Sommer regelmässig nach Wirtspflanzen abgesucht wurden. Zur Erhärtung der Felduntersuchungen wurden die hier gefundenen Wirtspflanzen im Gewächshaus in Töpfen mit gleichmässig verseuchter Erde noch einmal auf Anfälligkeit geprüft. Die Bonitierung des Schädigungsgrades erfolgte nach Augenschein in leichten, mittleren und schweren Befall.

Bei den Felduntersuchnungen zeigten Roggen (*Secale cereale* L.), Hafer (*Avena sativa* L.), Rüben (*Beta vulgaris* L.), Mais (*Zea mais* L.) und Sonnenblumen (*Helianthus annuus* L.) die stärksten Schäden, während bei Kartoffeln (*Solanum tuberosum* L.), Rotklee (*Trifolium arvense* L.) und Luzerne (*Medicago sativa* L.) nur leichte Befallssymptome aufzuweisen hatten.

Von den Unkrautarten waren am stärksten beschädigt: Ackergänsedistel (*Sonchus arvensis* L.), Ackergauchheil (*Anagallis arvensis* L.), Ackerhellerkraut (*Thlaspi arvense* L.), Ackersenf (*Sinapis arvensis* L.), Flohknöterich (*Polygonum persicaria* L.), Flughafer (*Avena fatua* L.), Hundspetersilie (*Aethusa cynapium* L.) und Windenknöterich (*Polygonum convolvulus* L.).

Mittelstarke Beschädigungen zeigten Ackerwinde (*Convolvulus arvensis* L.), Ausgebreitete Melde, Grosser Wegerich (*Plantago major* L.), Kleblabkraut (*Galium aparine* L.), Vogelknöterich (*Polygonum aviculare* L.) und Weisser Gänsefuss (*Chenopodium album* L.). Leichte Befallssymptome zeigten Ackerhahnenfuss (*Ranunculus arvensis* L.), Gemeiner Hohlzahn (*Galeopsis tetrahit* L.), Klatschmohn (*Papaver rhoeas* L.), Nachtlichtnelke (*Melandrium noctiflorum* Fr.) und die Vogelmiere.

Im Gewächshaus wurden neben den auf verseuchten Feldern vorgefundenen Wirtspflanzen noch die Unkräuter Bingelkraut (*Mercurialis annua* L.), Gemeines Kreuzkraut (*Senecio vulgaris* L.) und Pfeilkresse (*Lepidium draba* L.) in den Versuch eingeschlossen. Von den zwei Roggenformen stand hier nur die Winterform in Prüfung. Der Gemeine Hohlzahn wurde nicht mehr im Gewächshaus untersucht, da hier die Versuche bereits abgeschlossen waren, als Befall bei den Felduntersuchungen festgestellt wurde. Der Versuch zeigte, dass sämtliche geprüften Pflanzen, ausser dem Grossen Wegerich, von *Ditylenchus dipsaci* befallen wurden. Die Wirtspflanzen Rüben, Sonnenblumen, Ackergauchheil, Ackerhellerkraut, Ackersenf, Bingelkraut, Flohknöterich, Hundspetersilie, Kleblabkraut und Windenknöterich waren hier stark bzw. mittelstark beschädigt, während alle anderen Wirtspflanzen nur leicht angegriffen waren. Der stärkere Befall eben-

genannter Pflanzen dürfte auf eine grössere Empfindlichkeit gegenüber *Ditylenchus dipsaci* zurückzuführen sein.

Nicht befallen wurden im Feld- wie im Gewächshausversuch von den Kulturpflanzen Winterweizen (*Triticum aestivum*), von den Unkräutern Ackerdistel (*Cirsium arvense* Scop.), Gemeiner Erdrauch (*Fumaria officinalis* L.), Geruchlose Kamille (*Matricaria maritima* L.), Quecke (*Triticum repens* L.) sowie Windhalm (*Aspera spica venti* P.B.).

Bei Sommergerste (*Hordeum sativum* Jessen.) waren nur in einem Falle geschlechtsreife Tiere vorhanden, die sich jedoch nicht weiter vermehrten.

Schadbilder. Die Schadbilder sind je nach Pflanzenart verschieden. Der grösste Teil zeigt die normalen durch *Ditylenchus dipsaci* hervorgerufenen Krankheitssymptome: Aufplatzen des befallenen Gewebes, Missbildungen an Blättern und Stengeln und schliesslich Abfaulen des kranken Gewebes. Einige Pflanzen jedoch zeigten etwas abweichende Befallssymptome.

1. Nach Eindringen der Aelchen treten im Anfangsstadium der Entwicklung an den Stielen der Keim- sowie der ersten Laubblätter Verdickungen und leichte Nekrosen auf. Letztere sind immer nur oberflächlich vorhanden (Rotklee, Luzerne, Pfeilkresse und Nachtlichtnelke). In der Folgezeit verschwinden die Symptome.

2. An den Pflanzen treten nur ganz leichte, kaum bemerkbare Befallssymptome auf. Nekrosen sind nicht vorhanden. Bei Ackerhahnenfuss und Klatschmohn Anschwellen der Sprossbasis, bei Vogelmiere Einrollungen der Blätter und Verkürzung der Internodien.

3. Das Gewebe platzt nach Eindringen der Aelchen auf, jedoch trocknen die Befallsstellen bald ein, so dass es nicht zu tiefgreifenden Gewebezerstörungen kommt (Bingelkraut). Befallene Pflanzen zeigen stark gestauchten Wuchs und verkrümmte Blätter.

4. Es entstehen manchmal leichte Nekrosen, in den meisten Fällen jedoch nur Verbiegungen und Verkrümmungen der Blätter und Stiele, ohne dass Aelchen dort eingewandert sind (Ackerwinde).

Der Einfluss der Wirtspflanze auf die Grössenentwicklung von Ditylenchus dipsaci. Ludwig (1938), Stephenson (1942) und Goodey (1952) stellten bereits Grössenveränderungen bei Älchen fest, die auf das Kulturmedium bzw. die Wirtspflanzen zurückführen waren.

Um den Einfluss einer Anzahl Wirtspflanzen auf die Entwicklung

von *Ditylenchus dipsaci* zu untersuchen, wurden Nematoden aus sämtlichen oben genannten Wirtspflanzen ausser Sonnenblumen, Rotklee, Luzerne, Kartoffeln, Windenknöterich und Gemeinem Hohlzahn gemessen und mit denen aus Rüben verglichen. Die Aelchen wurden aus den Pflanzen nach der Baermannschen Trichtermethode gewonnen, bei 66° Celsius abgetötet und in Wasser auf den Objektträger gelegt. Die Messungen erfolgten mit einem Zeiss-Opton-W-Stativ. Aus Rüben wurden 40 Älchen gemessen, 20 Männchen und 20 Weibchen. Die Zahl der Messungen aus den übrigen Wirtspflanzen richtete sich nach dem Besatz an Nematoden und schwankte zwischen 18 und 40. Die statistische Auswertung erfolgte auf indirektem Wege über die Einzelwerte. Als statistische Sicherung wurde ein P-Wert kleiner als 0,1 angenommen. Gemessen wurde an jedem Älchen Körperlänge, Körperbreite und bei den Weibchen noch die Lage der Vulva in Prozent der Gesamtkörperlänge. Um den Einfluss der Verweildauer der Älchen in den Pflanzen auf die Grössenentwicklung möglichst zu verringern, wurden je Wirtspflanze vier Älchenproben entnommen. Die kleinsten Werte ergaben Älchen aus Weissem Gänsefuss, Vogelmiere, Klatschmohn, Ackersenf, Mais, Ackerhahnenfuss, Bingelkraut, Nachtlichtnelke und Pfeilkresse. Bei Älchen aus Klatschmohn, Ackerhahnenfuss und Nachtlichtnelke waren die Mittelwerte von Körperlänge und Körperbreite, im Vergleich zu Rüben, alle statistisch gesichert. Nicht mehr statistisch gesichert waren aus obengenannten Pflanzen die Mittelwerte von Körperbreite bei Männchen aus Senf, Mais, Pfeilkresse, Bingelkraut und bei Weibchen aus Weissem Gänsefuss und Bingelkraut. Ebenso waren nicht statistisch gesichert die Mittelwerte von Körperlänge bei Männchen aus Vogelmiere sowie bei Weibchen aus Bingelkraut. Die Lage der Vulva, ein wichtiges systematisches Merkmal, wurde vom Wirte nicht beeinflusst.

SUMMARY

For some years attacks of beet stem eelworm (*Ditylenchus dipsaci*) of such severity have occurred in parts of Baden-Württemberg as to render beet growing more or less uneconomic. Host range tests in the field and in glasshouses were done in 1954-57. With the exception of common thistle (*Cirsium arvense*), fumitary (*Fumaria officinalis*), couch grass (*Agropyron repens*) and silky Apera (*Apera spica-venti*), all weeds investigated were hosts of this local race of stem eelworm. Somewhat abnormal symptoms in red clover (*Trifolium pratense*), lucerne (*Medicago sativa*), corn crowfoot (*Ranunculus arvensis*), annual mercury (*Mercurialis annua*), field poppy (*Papaver rhoeas*), night flowering campion (*Melandrium noctiflorum*), hoary pepperwort (*Lepidium draba*), chickweed (*Stellaria media*) and bindweed (*Polygonum convolvulus*) point to a rather greater

resistance to this race. Red clover, lucerne and summer barley (*Hordeum sativum*), although invaded, do not appear to be true hosts as the eelworms do not breed in them. Attempts were made to correlate eelworm size with host plants and symptoms but larger numbers need to be measured before conclusions can be drawn.

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REVIEW OF THE GENUS *MELOIDOGYNE*

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Root-knot is one of the oldest known nematode diseases of plants. It has been serious and widespread for so long that an extensive literature has grown up on many aspects of the subject. I propose to deal here with some aspects of the taxonomy of the causal nematodes. The first recognizable record of root-knot disease was made over 100 years ago, when a disease of greenhouse cucumbers was described in a gardening journal in England (Berkeley, 1855). The roots of the plants bore numerous galls, within which were cyst-like bodies containing eggs and so-called "vibrios". Twenty years later galls due to nematodes were found on the roots of *Sempervivum tectorum* L. in Italy (Licopoli, 1875), and in 1878 Jobert described galls containing nematodes on the roots of coffee in Brazil. The nematodes associated with these galls were not closely studied at the time, but in 1879 Cornu, in the course of a study of *Phylloxera* on vines in France, observed on the roots of sainfoin (*Onobrychis viciifolia* Scop.) some yellow cyst-like bodies containing eggs and larvae, which he described and illustrated. He recognized that they were related to *Heterodera schachtii* Schmidt, 1871, which at that time was beginning to cause great damage to sugar beet in Germany, but, not wishing to place them in the same genus erroneously, he named them *Anguillula marioni*. A study of Cornu's description and illustrations shows that he had found a species of root-knot nematode and not a cyst-forming *Heterodera*.

Between 1879 and 1889 root-knot nematodes were described and named from a number of host plants in Java, Brazil and Florida, and in each case they were placed in a different genus. The confusion in taxonomy continued for nearly half a century until 1932, when Goodey published a paper explaining certain discrepancies. He made it clear that the specific name *radicicola*, which was then in general use for the root-knot nematodes, really applied to another nematode which was first described by Greeff in 1872 from galls on the roots

of grasses in Germany. This is the nematode now known as *Ditylenchus radicicola* (Greeff, 1872) Filipjev, 1936. Goodey considered that the root-knot nematodes should be included in the genus *Heterodera,* and since the earliest specific name given to a root-knot nematode was *marioni,* given by Cornu in 1879, he lumped them all together as *Heterodera marioni.*

This state of affairs was generally accepted for a time, but advances in the knowledge of host-parasite relationships soon led to the realization that root-knot nematodes could not be considered as comprising one single species. In order to understand how this realization grew it must be remembered that root-knot disease was a serious problem to agriculturalists in many parts of the world, mainly, though not only, in tropical and sub-tropical regions. In 1909 Marcinowski had listed 236 host plants and two years later Bessey gave 480. By 1938 there were over 1,300 recorded hosts (Buhrer, Cooper & Steiner 1933 and Buhrer 1938). The list has now grown to over 2,000 including such important crop plants as rice, maize, potato, tomato, clover, cotton, pineapple, peach, vines, tobacco, many vegetables and a great many ornamental trees, shrubs and herbaceous plants. Because of the serious losses caused by the disease, a great deal of work was done in attempts to find methods of control, while the nematodes themselves were at first given relatively little close study. It soon became obvious that no easy and cheap method of chemical control for use in the field would be found quickly, so a search for resistant crops was initiated. But when the subject of host range and resistance came to be considered it became clear that nematode populations from different areas sometimes behaved differently. For example, Christie & Albin (1944) in America tested nine populations of root-knot nematodes on *Trifolium resupinatum* L.: two multiplied successfully, three succeeded in reproducing, but in relatively small numbers, two entered the clover roots but did not come to maturity and two did not even enter the roots. In further experiments Christie (1946) found that other plants, such as cotton and peanuts, also reacted differently towards different populations of root-knot nematodes. Christie came to the conclusion that distinct biological races existed amongst the different populations of what was then known as *Heterodera marioni.* Then, in 1949, Chitwood published a paper which has radically changed the outlook of workers on this problem.

Chitwood was the first to make careful studies of the morphology of root-knot nematodes from different sources and to find differences

between them. He considered the differences to be of sufficient importance for some of the populations to be regarded as separate species. Furthermore, he concluded that the root-knot nematodes should be separated from the cyst-forming Heteroderas. CHITWOOD's work has led others to give careful attention to the morphology of root-knot nematodes, with the result that several new species have been described since 1949.

An examination of the pharynx and oesophagus of any stage of a root-knot nematode shows that it belongs to the family Tylenchidae, having a well-developed mouth stylet, close behind which may be seen the junction of the dorsal oesophageal gland duct with the lumen of the oesophagus. The anterior narrow region of the oesophagus is followed by a swollen muscular median bulb, and a glandular lobe overlies the beginning of the intestine ventro-laterally. All these characters are shared with *Heterodera*, as is also the marked sexual dimorphism, though this most obvious character differs in detail in the two groups. In root-knot nematodes (with the exception of one species) the swollen flask-shaped females always retain a thin colourless or white cuticle, and in most species the eggs are practically all deposited outside the body in a gelatinous matrix, while in *Heterodera* the female body wall forms a tough, brown envelope enclosing all, or at least half of the eggs. There are also important morphological differences in the structure of the head between the root-knot nematodes and the Heteroderas, while the males differ in that those of the root-knot nematode frequently have two testes, a character not known in any other phasmidian genus. In the female the excretory pore is much further forward in *Meloidogyne* than in *Heterodera*. The effect of parasite on host also differs in the two groups, the root-knot nematode, as is obvious from its name, usually, though not always, causes local enlargement of the host root, while this is rarely the case with *Heterodera*.

These differences convinced CHITWOOD that it was necessary to separate the root-knot nematodes from the cyst-formers and he revived the generic name *Meloidogyne* given by GOELDI (1887) to the nematodes found in galls on coffee roots in Brazil. CHITWOOD recognized five species and one variety, and descriptions of other species and varieties have since been made by other workers. At the present time eight species, two subspecies and one variety are known, namely:

Meloidogyne exigua Goeldi, 1887

M. javanica (Treub, 1885) Chitwood, 1949

M. javanica subsp. *bauruensis* Lordello, 1956
M. hapla Chitwood, 1949
M. incognita (Kofoid and White, 1919) Chitwood, 1949
M. incognita var. *acrita* Chitwood, 1949
M. arenaria (Neal, 1889), Chitwood, 1949
M. arenaria subsp. *thamesi* Chitwood, 1952
M. inornata Lordello, 1956
M. brevicauda Loos, 1953
M. acronea Coetzee, 1956.

Two species, *marioni* of Cornu and *vialae* of Lavergne (1901), were inadequately described, and it has so far been impossible to obtain material for a new study of them, or to decide whether they are good species or not.

The separation of the species is not easy, and yet it is important from the practical point of view because, as has already been indicated, the host ranges of the different species are not identical, a fact which can often be exploited by the agriculturalist to his advantage. Of the characters used by Chitwood for separating the species, one which has proved very useful and has received much attention is the cuticular pattern found at the posterior end of the mature female. This consists of striae on the cuticle and of what remains of the lateral line in the area around the vulva, anus and the site of the larval tail tip. It is referred to as the perineal pattern or the anal plate pattern. Other features used are the size and shape of the stylet and the position of the opening of the dorsal oesophageal gland duct in relation to the stylet base in the male, female and larva.

In an established infestation it is comparatively easy to remove females from the galls and to make mounts of the cuticle of the perineal region, while it is often impossible to find males. Great attention has therefore been given to the study of perineal patterns. Workers have at times overlooked Chitwood's statement that *Meloidogyne* is an extremely adaptable genus in which, he says "the morphological characters show considerable variation". He states, in fact, that he has not seen two identical specimens, but, he says, "Nevertheless general pattern series and other structures show similarities, and progenies from individual females are relatively consistent both as to morphology and host range". It is obvious therefore that although the perineal pattern is a useful character by which to identify species, one must be prepared for variations from the pattern typical of a species, and other morphological characters must be considered at the same time.

Before considering the variability of perineal patterns it is well to remind oneself of the structure of this area (Fig. 1).

The term "arch" is one which needs some explanation. CHITWOOD used it to describe the course of the striae in the region posterior to the anus. TAYLOR, DROPKIN & MARTIN (1955) use the "height" of this arch as a key character in identifying certain species, and it is therefore important to define it. DROPKIN (in litt.) has suggested that it be defined as follows: starting from the tail tip, measure outwards along each lateral line a distance equal to the vulva-anus distance. Then the arch is the stria joining these two points. This is quite a useful definition if the lateral lines can be traced with any certainty, but often, for example, in *incognita*, this is impossible. The two landmarks which are always visible in any specimen in good condition are the vulva and the anus. If a line is imagined at right angles to the vulva-anus axis, passing through the anus, the arch may be characterized by the ratio of the height above this line of the first complete arch-stria to the distance apart of its ends, where they cross the line. Height of arch is of course only one of several features which may be useful in describing the pattern of any particular species: others relate to the smoothness or otherwise of the striae, the character of the lateral field area and of the area which in the larva was the tip of the tail, and the presence and position of phasmids. For example, in *M. hapla* (Fig. 2) the arch is low and the tail often speckled: the lines are smooth and the position of the lateral lines may be marked by a change in direction of the striae. In *M. javanica* (Fig. 3) the lateral lines are exceptionally well marked and often double, and in *M. incognita* the striae form a high arch and are fine and much zig-zagged.

But it must be remembered that, as CHITWOOD pointed out, these patterns are variable. An attempt was made by DROPKIN (1953) to find some means of assessing the variability of the perineal pattern in populations originating from single larvae. He worked with several populations of *M. arenaria* and of *M. incognita* v. *acrita* and he assessed the variations in the patterns by means of tracings and measurements made from photographs. He could detect no differences in variability between a population of *arenaria* derived from a single larva and one of mixed ancestry. The coefficient of variability was 8 % and 12 % in two *arenaria* populations and from 8 %-13 % in four populations of *M. incognita* v. *acrita*. ALLEN (1952) also made observations on the variability of perineal patterns. In females originating from a single egg mass of *M. incognita* v. *acrita* he found in-

dividuals having well-marked lateral lines resembling those characteristic of *M. javanica* while others had a lateral line on one side only, or none at all. So far we have no information on variations in perineal patterns before as compared with after egg-laying, or variations shown by single-larva populations on different host plants, or developed under different growing conditions. Some examples of variability within families are shown in Figs. 4-7.

If one wishes to identify a population, the patterns shown by a number of individuals must be compared, and other characters must also be observed and taken into consideration. Although TAYLOR, DROPKIN and MARTIN, in their paper of 1955, give a key for the identification of six species, one subspecies and one variety of *Meloidogyne* by means of their perineal patterns, several new species have since been described and therefore it is now incomplete. Some of these recently described forms have characteristic perineal patterns but not all of them. LORDELLO (1956a) described *M. javanica* subspecies *bauruensis* found in Brazil on soybeans, which has a pattern resembling that of *javanica* but with a higher arch and more wavy lines. While *M. inornata,* also described by LORDELLO (1956b) from soybeans in Brazil, has a perineal pattern which may be confused with that of *M. incognita,* and this species, he says, cannot be identified by the pattern alone. In contrast to this, *Meloidogyne acronea* is quite distinctive in having no clear perineal pattern at all. This species, described from *Sorghum* in South Africa (COETZEE, 1956) has the vulva and anus situated on a protuberance. The cuticle is tough and the mature female may be exposed on the root surface and not embedded in a gall. These characteristics are strongly reminiscent of *Heterodera* and would serve to distinguish this species from other *Meloidogyne* species without reference to the cuticular pattern. Another aberrant population occurring in South Africa is one found on potatoes and described by SANDGROUND in 1922. In this species the eggs are said all to be retained within the female, and the larvae hatch and emerge after her death. The female cuticle is also distinctive, being dull instead of pearly white.

From *M. acronea* and SANDGROUND's species the next step leads to the truly intermediate genus *Meloidodera* (CHITWOOD ET AL 1956) in which no gall is formed in the host root, the female cuticle is tough, the vulva is about 70 % of the total length from the anterior end instead of being terminal and the eggs are retained in the female body. The cuticular pattern round the vulva is somewhat like that of *hapla*

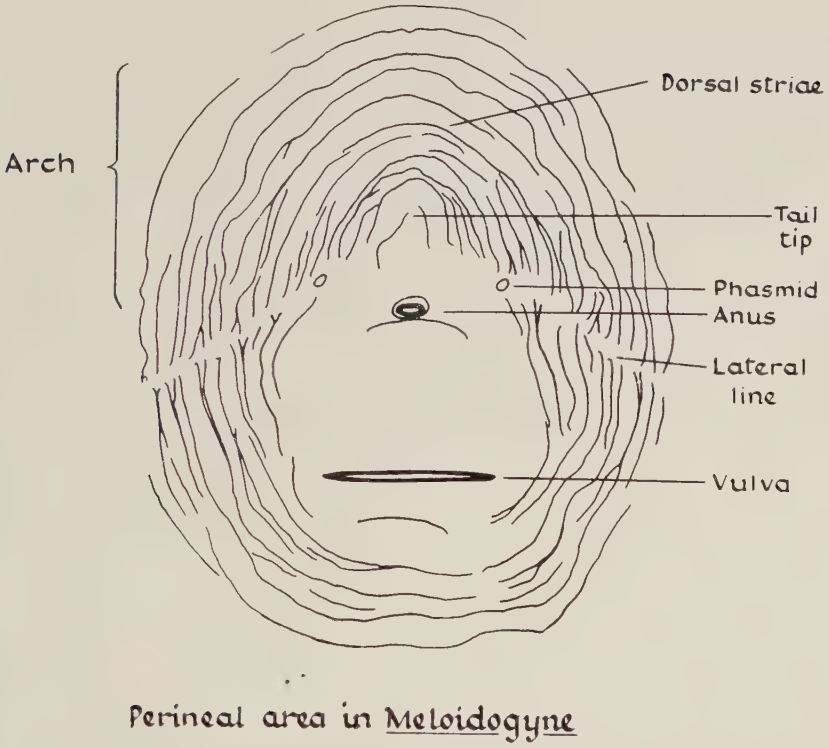

Fig. 1. Diagram of cuticular pattern in perineal region of *Meloidogyne*.

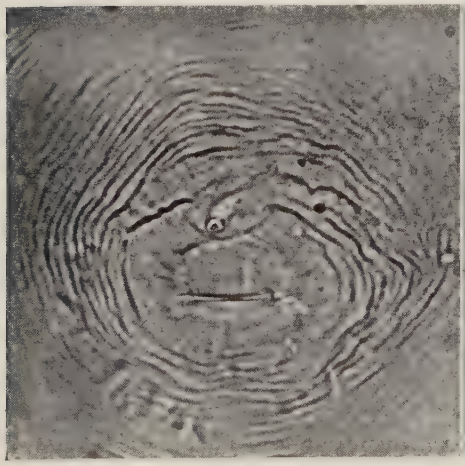

Fig. 2. Perineal pattern in *M. hapla*.

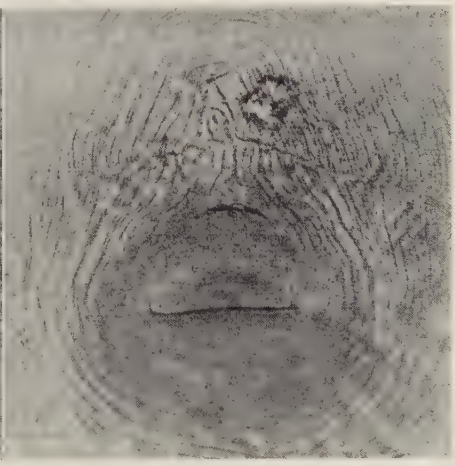

Fig. 3. Perineal pattern in *M. javanica*.

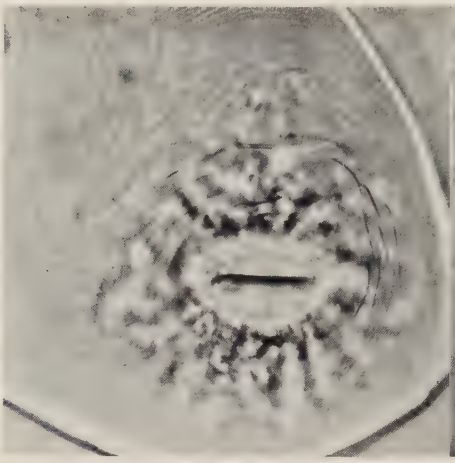

Fig. 4. Pattern in a daughter of the individual shown in Fig. 2. Note single "wing" on left side.

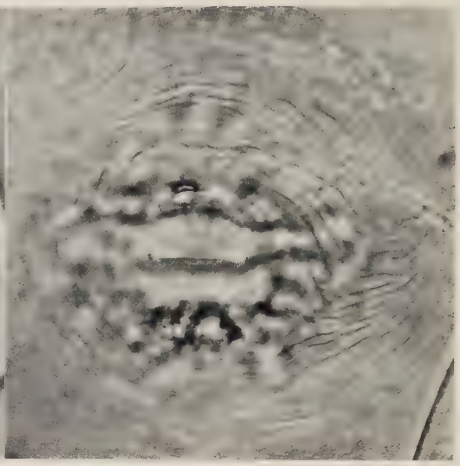

Fig. 5. Another daughter of individual in Fig. 2, showing "wing" on right side.

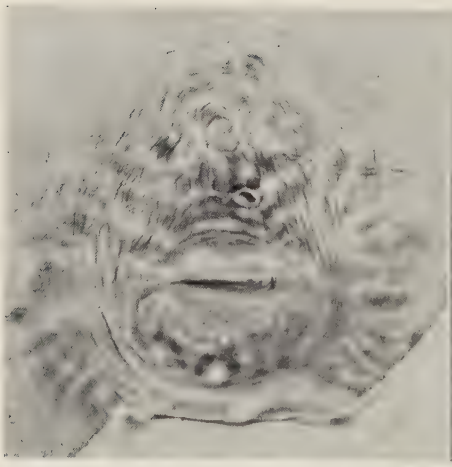

Fig. 6. Pattern in a daughter of the individual shown in Fig. 3, typical of *M. javanica*.

Fig. 7. Another daughter of the individual shown in Fig. 3. Atypical, with hardly distinguishable lateral lines.

but, owing to the much more anterior position of the vulva, the tail tip, lateral lines and anus are not involved.

Although, as already mentioned, the different species of *Meloidogyne* have many host plants in common, some species are unable to reproduce on certain plants. By a careful choice of plants it is possible to use host suitability as an aid to identification, and SASSER (1952) has worked out a scheme in which four species and one variety of *Meloidogyne* may be separated by the use of four plant species. This method, however, should be considered only as a verification of identification based on morphological characters, because it depends to some extent on negative results in infection tests and also on the variety of the test plant.

Evidence has recently appeared of variations in host preference within root-knot nematode species. Examples have been given by VAN DER LINDE (1957) in South African populations of *M. incognita* v. *acrita*. He studied the degree of attack on upland cotton (*Gossypium hirsutum* L.) of nematode populations from nine different localities. One population produced a few egg masses, six populations invaded the roots but did not reproduce and two failed to penetrate the roots. On two varieties of velvet bean (*Stizolobium deeringianum* Bort., varieties Velvet Golden and Velvet Stingless) the different populations varied between failure to penetrate by two or three populations, apparently normal penetration and reproduction by five or six populations and intermediate behaviour by some others. The situation is complicated by the fact that the host plant variety is important: with a third variety, Velvet Somerset, VAN DER LINDE found that larvae of all the nine nematode populations penetrated the roots but two failed to reproduce and three reproduced in only very small numbers.

Other workers have found varietal differences in other crop plants: for example, certain varieties of cotton showed resistance to *M. incognita* and *M. incognita* var. *acrita* (WILES, 1957), certain varieties of maize showed resistance to *M. incognita* (NELSON, 1957), and varieties of lima bean, soybean, oats and red pepper (*Capsicum*) showed resistance to *M. incognita* var. *acrita* (ALLARD 1954, CRITTENDEN 1954 and 1956, HARE 1956). In these cases apparently only a single population was used to represent each nematode species or variety. In view of VAN DER LINDE's results it seems advisable to use several populations of the parasite from different sources. While populations may differ only biologically, a close examination may show that they also have morphological differences.

This situation is well demonstrated by LORDELLO's work. He observed that in the Bauru region of the State of São Paulo, in Brazil, the soybean variety La 41-1219, amongst others, was attacked by the root-knot species *M. incognita.* The same variety, when grown in the Campinas region was resistant to a nematode having a perineal pattern closely resembling that of *M. incognita,* while other varieties were susceptible to it. When LORDELLO examined this nematode he found that it had morphological differences from *M. incognita* sufficiently marked to cause him to describe it as a new species, *M. inornata.* The chief differences between *M. incognita* and *M. inornata* are the greater width of the eggs in *M. inornata* (37.5-60 μ as compared with 30-38 μ), the possession of a single head annule in the larva and male as compared with 3 in *M. incognita,* and the more posterior position of the excretory pore in the female (at the 17th-22nd annule instead of about the 12th-14th). It should be remembered in this connection that ALLEN (1952) illustrates heads of male *M. incognita acrita* in which the number of head annules is variable. This emphasizes the importance of examining all possible stages of these nematodes, and the dangers of relying on one feature alone for identification.

It seems very probable that, as CHITWOOD has said "Members of the genus *Meloidogyne* are extremely adaptable and their morphological characters show considerable variation." Before identifying a root-knot nematode or designating a new species it is therefore very important to know the range of variation to be found within the well-established species. This is especially so if the characters used for differentiation are minute and are either dependent on measurements, such as the distance behind the stylet base of the opening of the dorsal oesophageal gland duct, or on a subjective assessment of shape, such as the form of the stylet knobs or the perineal pattern.

Although the situation has been greatly clarified by the work of CHITWOOD, there remain resemblances to the position as shown by CHRISTIE and his co-workers in the early 1940s, when different populations of what was then thought to be a single species behaved in different ways towards the same host plants. The taxonomist's arguments over apparently trivial morphological details, and his insistence on knowing as much as possible about the variability of a species as well as about its non-variable characters, may seem unnecessary to the practical man who is interested primarily in the success of his crops. But so long as the cultivation of resistant crops seems to be the only practical means, on a field scale, of avoiding serious losses from root-

knot disease, it is essential to be able to recognize the particular nematode against which resistance is required, and for this purpose the minute, and sometimes variable, morphological details are of fundamental importance.

ZUSAMMENFASSUNG

Die erste Beschreibung eines Nematoden, der jetzt als *Meloidogyne* bekannt ist, stammt von Cornu 1879, der ihn in Gallen von *Onobrychis sativa* in Frankreich fand. Die meisten früheren Beobachtungen über Wurzelgallenälchenbefall kamen aus tropischen und subtropischen Gebieten oder aus Gewächshäusern der gemässigten Zonen. 1932 gab Goodey einen Überblick über die Taxonomie der Wurzelgallennematoden und schloss sie alle zu einer Art, *Heterodera marioni,* zusammen. Bei Untersuchungen mit dem Ziel einer Bekämpfung des Schadens zeigten sich biologische Unterschiede zwischen den Populationen aus verschiedenen Gebieten. Dies veranlasste B. G. Chitwood, die Morphologie näher als bisher geschehen, zu überprüfen. Als Ergebnis kam er zu dem Schluss, dass diese Nematoden von den Heteroderen getrennt werden sollten. Er setzte die Gattung *Meloidogyne* wieder ein und gab neue Beschreibungen von fünf Arten und einer Varietät. Seitdem Chitwood's Arbeit veröffentlicht war, haben andere Forscher neue Arten und Unterarten beschrieben. Elf Formen sind jetzt bekannt. Die hauptsächlichen Merkmale, die zur Charakterisierung der Arten benutzt werden, sind Grösse und Form des Stachels beim Männchen und bei der Larve, die Lage der Verbindung der dorsalen Oesophagusdrüse mit dem Oesophagus und die Kutikularmusterung des Perineums. Die letztere variiert beträchtlich innerhalb der Arten, wie die beigefügten Abbildungen ergeben, während sie bei wenigstens zwei Arten nicht deutlich voneinander unterschieden ist. Der Wirtspflanzenkreis ist eine nützliche Hilfe bei der Identifizierung der Arten, aber es ist bereits gefunden worden, dass wenigstens eine Art von *Meloidogyne* Rassen zu enthalten scheint, die eine unterschiedliche Wirtspflanzenbevorzugung besitzen. Die Identifizierung von *Meloidogyne*-Arten ist also noch schwierig und unsere Kenntnis über die Gattung als Ganzes ist vielleicht noch weit davon entfernt, vollständig zu sein.

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DISCUSSION

J. H. Schuurmans Stekhoven fragt ob es möglich ist festzustellen, welche von den von Chitwood und anderen beschriebenen Arten mit der von Cornu als *marioni* beschriebenen Art identisch ist. Wenn das möglich sein würde, denn würde der Diskussionsredner es bevorzugen, *Meloidogyne marioni* für diese Art wiederherzustellen.

Mary T. Franklin: It has not been possible to identify Cornu's species *marioni*.

M. Ritter: Je n'ai pas pu retrouver de *Meloidogyne* dans la localité type: Chateauneuf sur Loire où le sainfoin (*Onobrychis sativa*) est très peu cultivé actuellement. Par contre des foyers endémiques de *Meloidogyne* sont connus près d'Orléans ainsi que dans la vallée de la Loire jusqu' à son embouchure. L'espèce me semble être *M. arenaria* et il est vraisemblable qu'elle correspond à celle décrite par Cornu sous le nom de *marioni*. Cependant, en l'absence de certitude il semble préférable, comme le souhaite B. G. Chitwood lui-même, de ne pas chercher à ressusciter le nom de *marioni*. Il est inutile de créer une nouvelle confusion dans une terminologie maintenant bien admise.

M. Oostenbrink: From the photographs and scientific indications in Russian publications we may conclude that there the name *Meloidogyne marioni* is in use. I have not been able to decipher with certainty which of the species mentioned by Miss Franklin is meant by this name.

H. Goffart: Es wird die grosse Variabilität besonders bei *M. arenaria* erläutert und auf die Ausbildung der dorsalen Kutikularlinien hingewiesen, die teils gewölbt, teils flach, teils eingesunken sind.

P. A. A. Loof: In the genus *Pratylenchus* some asexually reproducing species are very variable and difficult to delimit whereas normally reproducing ones form well characterized units. Do similar differences between mono- and bisexual species exist within the genus *Meloidogyne* as well?

Mary T. Franklin: Asexual reproduction is known to occur commonly in *Meloidogyne,* but it is not known in which species it occurs nor whether it occurs in those species in which males are common.

liert. Die Lufttemperaturen der verschiedenen Pflanzengruppen wurden mittels eines Thermographen registriert. Nur ausnahmsweise stieg bei den Gruppen B und C die Lufttemperatur wegen zu starker Sonnenstrahlung einige Stunden über die kritische Temperatur von 15° C.

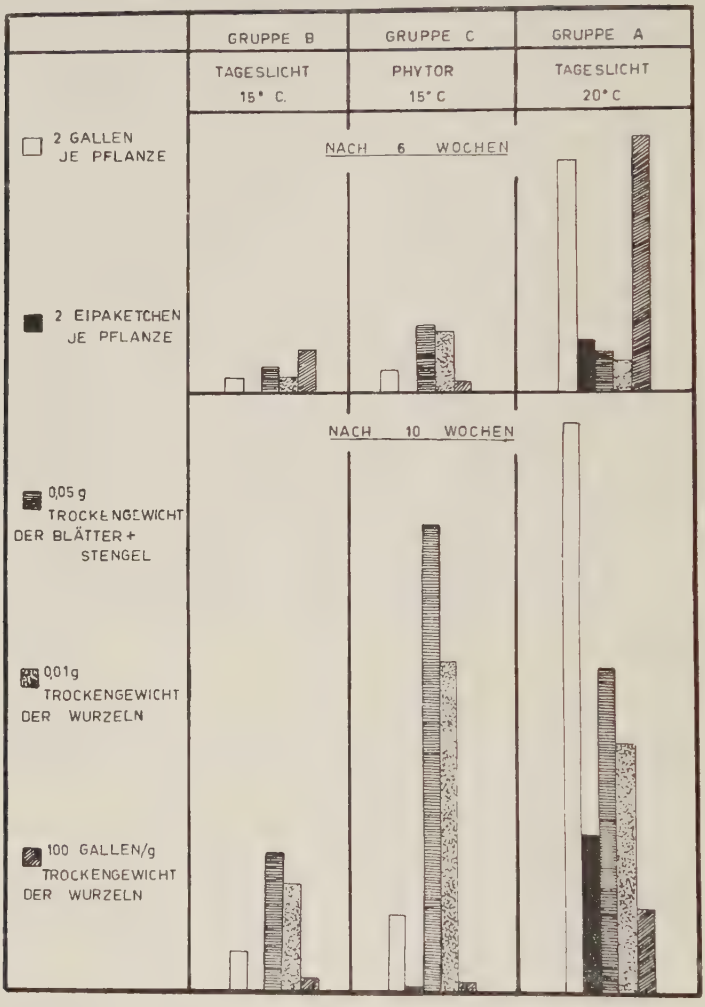

Abb. 2. Schematische Darstellung der Ergebnisse.

Anderseits muss betont werden, dass die Bodentemperatur der Lufttemperatur nicht so schnell folgt und geringeren Schwankungen unterworfen ist.

Die Hälfte (nämlich 3 × 11) der Pflanzengruppen wurde nach 6 Wochen, die andere Hälfte (nämlich 3 × 10) nach 10 Wochen ana-

lysiert. Es wurden bestimmt die Anzahl der Gallen, die Anzahl der Eipaketchen (zweite Generation), das Trockengewicht [1]) der „Blätter + Stengel" und das Trockengewicht der Wurzeln. Schliesslich wurde die Anzahl der Gallen je g Trockengewicht der Wurzeln berechnet.

VERSUCHSERGEBNISSE

Die Befunde der Untersuchungen sind aus Tabelle I ersichtlich, während sie graphisch in Abb. 2 dargestellt wurden. Die Abbildungen 3, 4 und 5 zeigen uns Aufnahmen der 3 Pflanzengruppen 6 und 10 Wochen nach Beginn des Versuches.

DISKUSSION UND SCHLUSSFOLGERUNGEN

Vergleichen wir in der Darstellung (Abb. 2) die Säulen der „Tageslicht-15° C"-Pflanzen mit denen der „Phytor"-Pflanzen, so kommt der Einfluss der künstlichen Beleuchtung zum Ausdruck. Vergleichen wir die Säulen der „Tageslicht-15° C"-Pflanzen mit denen der „Tageslicht-20° C"-Pflanzen, so tritt der Temperatur-Einfluss deutlich zum Vorschein. Was uns aber am meisten interessiert, ist der Vergleich zwischen den „Tageslicht-20° C"-Pflanzen und den „Phytor"-Pflanzen. Es hat sich herausgestellt, dass die „Phytor"-Pflanzen sich schöner und stärker entwickelt hatten (vergl. auch Abb. 3-5). Das Trockengewicht der Wurzeln und der „Blätter + Stengel" war erheblich höher und die Blätter zeigten eine dunklere Farbe. Anderseits war die Gallenzahl bei den „Phytor"-Pflanzen viel niedriger. Die Gallenzahl je g Trockengewicht der Wurzeln war auch ganz zu Gunsten der „Phytor"-Pflanzen. Hätte die Temperatur der „Phytor"-Pflanzen niemals die kritischen 15° C überschritten, so wäre der Unterschied hier gewiss noch frappanter gewesen.

Nach 6 Wochen hatten die *Meloidogyne*-Weibchen bei den „Phytor"-Pflanzen noch keine zweite Generation (= Eipaketchen) erzeugt, während bei den „Tageslicht-20° C"-Pflanzen im Durchschnitt 5,81 Eipaketchen je Pflanze beobachtet wurden. Bei der „Phytor"-Gruppe wurden insgesamt nur 2 Eipaketchen gefunden, gegenüber 230 [2]) bei der anderen Gruppe. Nach 6 Wochen erwiesen 4 der 11 „Phytor"-Pflanzen sich völlig nematodenfrei, während alle „Tageslicht-20° C"-Pflanzen stark durch Nematoden befallen waren.

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1) Um das Trockengewicht der „Blätter + Stengel" und der Wurzeln zu ermitteln, wurden diese bei 105° C bis zur Gewichtskonstanz getrocknet, abgekühlt und dann gewogen.

2) Nämlich 64 Eipaketchen nach 6 Wochen (11 Pflanzen) und 166 Eipaketchen nach 10 Wochen (10 Pflanzen).

TABELLE I

Ergebnisse der Untersuchungen

	Nach 6 Wochen			Nach 10 Wochen		
	Tageslicht 15° C	Phytor 15° C	Tageslicht 20° C	Tageslicht 15° C	Phytor 15° C	Tageslicht 20° C
Anzahl der untersuchten Tomatenpflanzen	11	11	11	10	10	10
Durchschnittl. Anzahl der nematodenfreien Pflanzen	4	4	0	1	1	0
Durchschnittl. Anzahl der Gallen je Pflanze	1,81	2,36	26,4	4,2	8,4	63,6
Durchschnittl. Anzahl der Eipaketchen je Pflanze	0	0	5,81	0	0,2	16,6
Durchschnittl. Trockengewicht der „Blätter + Stengel" je Pflanze in g.	0,0625	0,1900	0,1107	0,3759	1,3644	0,8936
Durchschnittl. Trockengewicht der Wurzeln je Pflanze in g.	0,0085	0,0345	0,0183	0,0591	0,1931	0,1388
Durchschnittl. Gallenzahl je g. Trockengewicht der Wurzeln	212,9	68,4	1442,7	71,0	44,0	458,2

J. VAN DEN BRANDE & A. GILLARD: *Versuch mit Meloidogyne hapla*

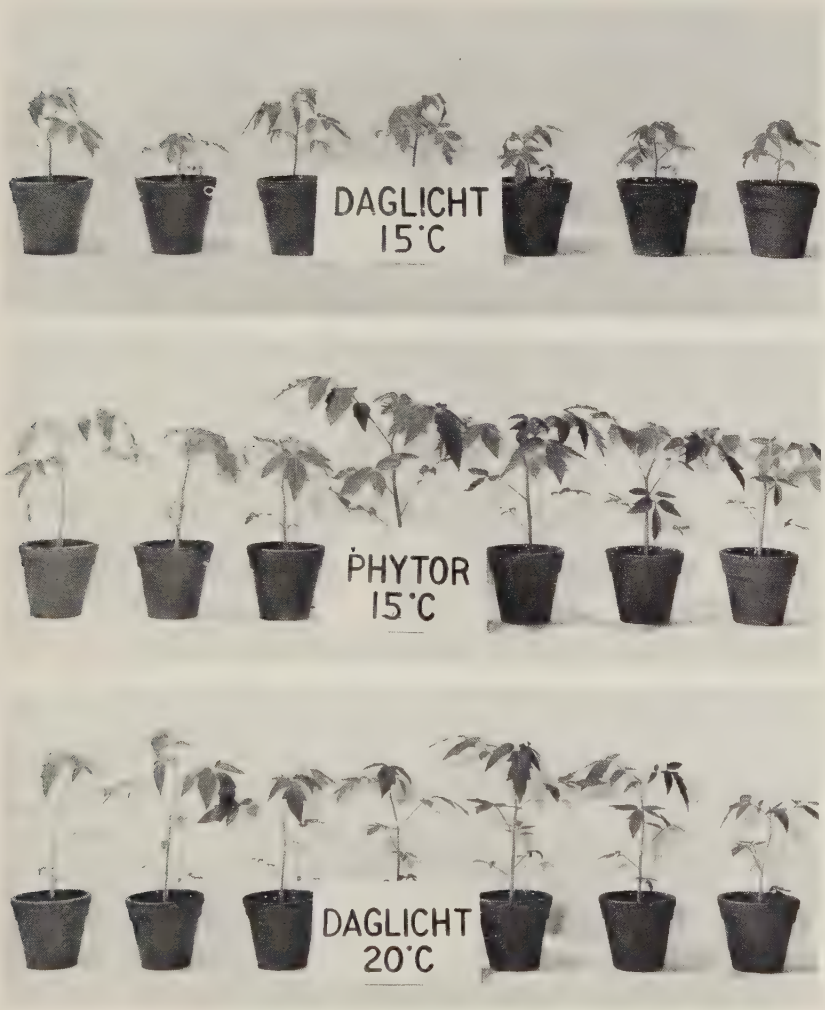

Abb. 3. Aufnahme der 3 Pflanzengruppen (Tageslicht 15° C, Phytor 15° C und Tageslicht 20° C) 6 Wochen nach Beginn des Versuches.

Abb. 4. Aufnahme von oben: die 3 Pflanzengruppen 6 Wochen nach Beginn des Versuches.

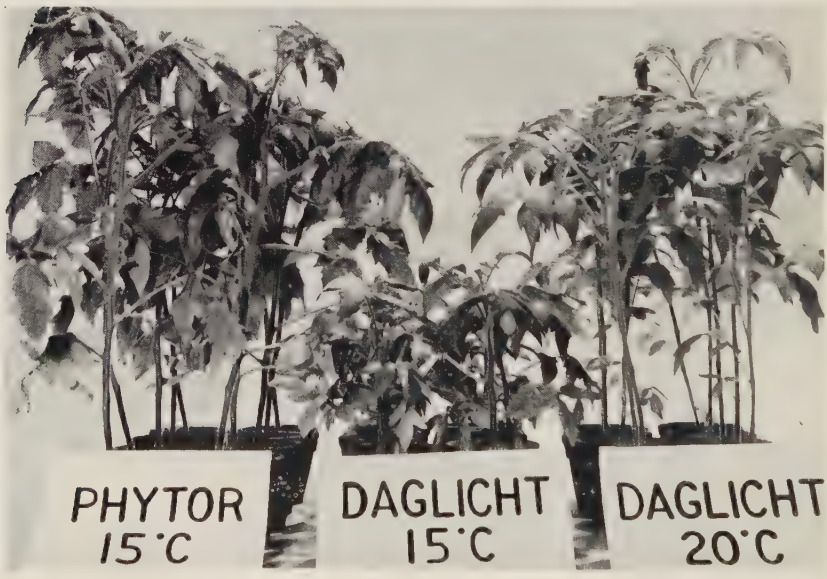

Abb. 5. Aufnahme der 3 Pflanzengruppen 10 Wochen nach Beginn des Versuches.

Die Schemata lassen also unschwer erkennen, dass man durch künstliche Beleuchtung und Züchtung der Pflanzen unter der kritischen 15° C-Temperatur ebenso schöne, sogar schönere Pflanzen züchten kann, die praktisch nematodenfrei sind. Unseren Befunden gemäss wurden nach 6 und 10 Wochen 95,3 % beziehungsweise 90,4 % der Wurzelgallenälchen durch die niedrigen Temperatur-Verhältnisse völlig in ihrer Entwicklung gehemmt. Züchtung der „Phytor"-Pflanzen unter 13° C hätte vielleicht 100 % der Wurzelgallenälchen gehemmt.

Es scheint also eindeutig erwiesen, dass die Nematoden durch Regulierung der Faktoren Licht und Temperatur in Schach gehalten werden können, ohne das Pflanzenwachstum zu benachteiligen. Das Streben zur Züchtung parasitenfreier Pflanzen durch Regulierung ökologischer Faktoren (wie Temperatur, Licht, CO_2 usw.) könnte man „Ökologischen Pflanzenschutz" nennen. Ob diese Methode für die Praxis (z.B. für Gewächshäuser) Interesse bietet, müssen weitere Versuche klarlegen.

SUMMARY

The authors tried to grow plants, free from nematodes, on root-knot infested soil by regulating light and temperature without using chemical, physical or biological control measures.

Sixty three tomato seedlings were planted in pots with heavily infested soil, divided into three groups of twenty one plants each, and grown in a glass house. Groups A and B received normal daylight. For Group A the temperature was kept around 20° C, for Group B around 15° C maximum. Group C received artificial light from 3 a.m. till 11 p.m., using 5 "Phytor" lamps, the temperature being kept just below 15° C. Only exceptionally, because of sunshine, did the temperature rise above the critical point of 15° C for a few hours a day in groups B and C.

The first half of the plants in each group was examined after six, the second half after ten weeks. The number of galls per plant, the number of egg masses, the dry weight of "leaves + stem", the dry weight of the roots and the number of galls per g. of dry root were determined.

The "illuminated" plants were the best developed of the three groups, the dry weights of root and "leaves + stem" being markedly higher. The number of galls per plant on the other hand was much lower in the "illuminated" group (2.36 after six weeks and 8.4 after ten weeks) than in the "daylight-20° C" plants (26.4 after six weeks and 63.6 after ten weeks). The number of galls per g. of dry root was also more favourable for the "illuminated" plants (68-1442 respectively after six weeks, 44-458 after ten weeks).

After six weeks, no egg masses had yet been produced by the *Meloidogyne* females on the "illuminated" plants, whilst on the "daylight-20° C" plants an average of 5.81 egg masses were found per plant.

After six weeks, four of the eleven "illuminated" plants were completely free from nematodes, whilst all the "daylight-20° C" plants were heavily infested. After six and ten weeks 95.3 % and 90.4 % respectively of the root-knot nematodes were completely checked in their development by the low temperature conditions.

The results might have been more striking had the temperature never risen above the critical 15° C for the "illuminated" plants, which if grown below 13° C would perhaps have been completely free from nematodes. Attempts to grow plants free from parasites by regulating ecological factors may be termed "ecological plant protection".

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DISKUSSION

B. HOMEYER (Köln, Deutschland). Unterschiedliche Befallsgrade liessen sich bei *Meloidogyne arenaria* und *M. incognita* bei 24° C durch Beleuchtung mit verschiedenen Wellenlängen erreichen. Bei normalen Leuchtstoffröhren war die Gallenbildung am geringsten, bei HPL-Leuchten am grössten. Es wird für möglich gehalten, dass bei der vorgetragenen Beobachtung die benutzten Wellenlängen des Lichtes von primärer Bedeutung ist.

ANTWORT: Auch unsere früheren Untersuchungen mit *M. arenaria* (*Nematologica,* **1** (3), 1956, 184-188) zeigten uns dass der Befallsgrad von den benutzten Wellenlängen abhänglich ist. Hier aber ist die Temperatur der limitierende Faktor für die Älchenentwicklung. Nicht nur die „Phytor-15° C-Pflanzen", sondern auch die „Tageslicht-15° C-Pflanzen" zeigten einen analogen niedrigen Befallsgrad.

J. GRAINGER (Auchincruive, Great Britain). This result brings a new and interesting point of view. Our own work at Auchincruive with fungal diseases (e.g. *Phytophthora,* potato blight) has proved a relation between severity of attack and a ratio within the plant denoting its potential of carbohydrate nutrition for the parasite (Grainger: *Phytopathology,* **46**, 1956). We thought it did not apply to eelworm diseases, but the present results show that growth of the host, and presumably its carbohydrate status, are involved in the severity of eelworm attack. Other results presented at this symposium combine with these to prompt a detailed examination of host physiology in relation to eelworm attack.

R. D. WINSLOW (Rothamsted, England). (In connection with Dr. Grainger's comments). Surely this is a direct temperature effect on the nematodes; the plants, due to the altered lighting conditions, are able to develop at a temperature too low for the development of the nematodes. A similar effect was found by Chitwood in America, where a variety of potato which begins growth early in the spring, i.e. at a low temperature, escapes damage by *Heterodera rostochiensis.*

M. OOSTENBRINK, (Wageningen, Holland). The thing to be explained in the trials shown is therefore not why there is no root-knot infestation in the treated plants (which will be due to the low temperature of 15° C), but why "Phytor" light increased the growth of the plants in comparison to daylight.

ANSWER: The ACEC-Phytor tubes were especially constructed to stimulate plant growth. They possess a very suitable emission spectrum. It is well-known that the photoperiod determines photosynthesis and that the composition of the emission spectrum influences photosynthesis, determines the habit of the plant and controls flowering.

Nematologica II, Suppl. (1957) : 405 S — E. J. Brill, Leiden.

CROP DAMAGE BY NEMATODES IN ISRAEL

BY

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(SUMMARY)

A review of various groups of nematodes which attack and damage crop plants in Israel, was given. Damage due to root-knot nematode (*Meloidogyne* spp.) was described on crops grown under irrigation, viz.: tomatoes, potatoes, strawberries, tuberoses, and bananas.

Most banana plantations are infested by spiral nematodes (*Helicotylenchus multicinctus* Golden, *H. nannus* Steiner and *Rotylenchus* sp.) which cause great reduction in growth vigor and yield.

Cyst-forming nematodes (*Heterodera* spp.) are apparently wide-spread in Israel: damage occurs to potted cacti (*H. cacti* Filipjev & Stekhoven) and wheat was heavily infested in some parts of the country by *H. major* Schmidt.

Some successful experiments on nematode control were mentioned. Trials on the freeing of planting material nematodes by the use of a relatively non-phytotoxic fumigant are in progress. It is hoped that clean suckers of bananas from fumigated plantations will be obtained. In the same way we hope to obtain clean planting materials of strawberries, tuberoses and gladioli.

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Nematologica II, Suppl. (1957): 406-412 S — E. J. Brill, Leiden

NEUERE GESICHTSPUNKTE ZUR FRAGE DER BIOLOGIE UND ÖKOLOGIE DER WANDERNDEN WURZELNEMATODEN

VON

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Im folgenden Referat soll versucht werden, einen kurzen Überblick über den heutigen Stand der Erforschung der sogen. migratory root nematodes zu geben. Einige besondere Fragestellungen werden dabei an Hand eigener Versuchsergebnisse und Beobachtungen eingehender besprochen.

Die Betrachtung wird sich auf europäische Verhältnisse beschränken. In Amerika ist zwar die nematologische Forschung in Vielem weiter als hier, aber es hat sich erwiesen, dass die dort gewonnenen Erkenntnisse nicht ohne weiteres auf Europa übertragen werden können. Die Nematodenschäden sind dort umfangreicher und vielseitiger. Bei uns unwichtige Formen, wie *Tylenchorhynchus,* richten in Amerika grosse wirtschaftliche Schäden an. Für diese Unterschiede dürften neben klimatischen Faktoren im wesentlichen ackerbauliche Massnahmen, wie enggestellte Fruchtfolgen, mangelnde Humusversorgung und künstliche Bewässerung verantwortlich sein (Goffart & Scheibe 1956). Nachdem in Amerika schon seit längerer Zeit über die wandernden Wurzelnematoden intensiv gearbeitet wurde, hat in den letzten Jahren auch in Europa eine eingehende Untersuchung dieser Schädlinge eingesetzt. Eine Reihe bisher ungeklärter Schäden an den verschiedensten Kulturpflanzen konnte auf Nematoden zurückgeführt werden.

Die wirtschaftliche Bedeutung dieser Älchen kann nicht mehr bezweifelt werden. Trotzdem stehen wir, verglichen mit den jahrzehntelangen Untersuchungen über die altbekannten Parasiten, wie *Heterodera* und *Ditylenchus,* erst am Anfang der Erforschung der Biologie dieser Nematoden, und es ist verständlich, dass sich auch über die Art der Schädigung verschiedene Meinungen bildeten. Fasst man die Literatur über die phytopathologische Bedeutung der wandernden Wurzelnematoden zusammen, so finden sich drei Hauptmeinungen:

1. Die Nematoden allein rufen die Schäden hervor. Andere Faktoren spielen keine Rolle.

2. Die Nematoden befallen zwar als Primärparasiten die Pflanzen, aber die eigentlichen Schäden treten erst auf, wenn andere Organismen hinzutreten.
3. Die Nematoden sind gar keine Primärparasiten, sondern nur Folgeschädlinge. Die eigentliche Ursache der Schäden sind andere Faktoren biotischer oder abiotischer Art.

Untersucht man, wie es zu der Verschiedenheit der Ansichten kam, so ergibt sich eine ganze Reihe von Gründen. Den häufigsten Anlass zu Meinungsunterschieden gibt ohne Zweifel die Anwendung verschiedenartiger, z.T. sehr ungleichwertiger Arbeitsmethoden und die unterschiedliche Bewertung von Symptomen. Dieses Problem ist Gegenstand des nächsten Vortrages (PITCHER), so dass es hier ausser Acht gelassen werden kann.

Grössere Schwierigkeiten macht auch die Unsicherheit in der Taxonomie. Die Gattungen sind zwar meist schon lange bekannt, aber die alten Artbeschreibungen sind oft ungenügend. Es sei nur an die Gattung *Pratylenchus* erinnert. Vor 5 oder 6 Jahren herrschte eine beachtliche Verwirrung über *Pratylenchus*-Arten. Im Jahre 1953 führten dann SHER & ALLEN eine Revision durch und stellten zu den 7 bzw. 8 bis dahin beschriebenen Arten 3 neue auf. Seitdem sind bereits wieder 5 neue Formen beschrieben worden (MEYL 1953, 1954; LORDELLO, ZAMITH & BOOCK 1955; TAYLOR & JENKINS 1957), und trotzdem ist das Artenproblem noch nicht restlos geklärt, wie der Vortrag von LOOF gezeigt hat.

Ferner wird leicht übersehen, dass die wandernden Wurzelnematoden zwar in ökologischer Hinsicht eine gewisse Einheit bilden, dass sie aber zu ganz verschiedenen taxonomischen Gruppen gehören und sich in wesentlichen Punkten ihrer Lebensweise stark unterscheiden. Ein gutes Beispiel dafür sind *Pratylenchus* und *Paratylenchus*. Paratylenchen sind im allgemeinen träge Tiere, die meist längere Zeit an einer Stelle saugen. Sie stechen in der Regel nur die Zellen der Wurzelepidermis an und dringen selten tiefer ein.

Pratylenchen dagegen sind lebhafter, kriechen an den Wurzeln entlang und verletzen sie an vielen Stellen. Ausserdem findet man sie meist tiefer im Wurzelgewebe. Einer der wesentlichsten Unterschiede ist aber der verschiedenartige Einfluss, den die beiden Nematoden auf das Pflanzengewebe ausüben, wie eigene Versuche gezeigt haben. Hängt man die Wurzeln einer gesunden Möhre in eine 0,5 % Lösung von Rhodamin B, so wird der Farbstoff aufgenommen und

durch den Zentralzylinder weitergeleitet. Das Parenchymgewebe färbt sich nicht an. Nimmt man aber eine Wurzel, die von *Pratylenchus* befallen ist, dann tritt der Farbstoff an den Stellen, an denen Älchen sitzen, aus dem Gefässbündel in das umgebende Gewebe. In der Nähe der Nematoden ist also die Permeabilität des Gewebes verändert. Bei *Paratylenchus* dagegen bleibt alles unverändert. Der Farbstoff tritt nicht in das Parenchymgewebe ein. Daraus ist zu schliessen, dass *Paratylenchus* hauptsächlich durch mechanische Verletzung der Wurzeln und damit Schwächung der Pflanze schädlich ist. Bei *Pratylenchus* kommt noch ein chemischer Einfluss hinzu. Diese Annahme wird unterstützt durch den Befund, dass bei *Paratylenchus*-Befall nur selten Nekrosen auftreten, bei *Pratylenchus* dagegen regelmässig. Auch die Höhe der kritischen Befallszahl macht den Unterschied zwischen den beiden Gattungen deutlich. Darunter ist die Anzahl Nematoden in einer bestimmten Bodenmenge zu verstehen, die an einer in diesen Boden gebrachten Pflanze sichtbare Schäden verursacht. Wir haben an Möhren bei einer Ausgangspopulation von etwa 150 *Pratylenchus pratensis* De Man in 250 ccm Erde die ersten sichtbaren Schäden festgestellt. Bei *Paratylenchus* war das erst bei einer Ausgangspopulation von etwa 1500-2000 Tieren zu beobachten. Der Wert für *Hoplolaimus*, einem anderen wichtigen Schädling, liegt bei Blumenzwiebeln nach Slootweg (1956) bei etwa 500 Tieren in 1 kg Boden. Solche Verseuchungsgrade sind, gemessen an den kritischen Befallszahlen anderer Parasiten, wie z.B. *Ditylenchus dipsaci* (Kühn) Filipjev, sehr hoch. Genügen doch schon 4-5 Stockälchen in 500 g Boden, um an empfindlichen Wirtspflanzen sichtbare Schäden hervorzurufen (Seinhorst, 1956). In derartig geringer Anzahl findet man wandernde Wurzelnematoden praktisch in jeder Bodenprobe von landwirtschaftlich oder gärtnerisch genutzten Flächen, ohne dass ein Zusammenhang mit Schäden zu bestehen braucht. Rein qualitative Untersuchungen über das Vorhandensein sind daher bei diesen Nematoden unzulänglich und können leicht zu Fehlschlüssen führen.

Wichtig ist auch die Beachtung der jeweiligen Pflanzenart. Eine strenge Wirtsspezialisierung findet man zwar in der Regel nicht, wohl aber eine gewisse Bevorzugung. So kommt nach unseren Beobachtungen z.B. *Hoplolaimus* besonders an Gemüse vor, während er durch Getreide stark gedrückt wird. *Pratylenchus pratensis* dagegen ist besonders an Getreide und *P. penetrans* (Cobb) Sher & Allen an Baumschulgewächsen zu finden, ohne dass man deshalb etwa von getrennten Wirtspflanzenkreisen sprechen könnte. Ausserdem muss man unter-

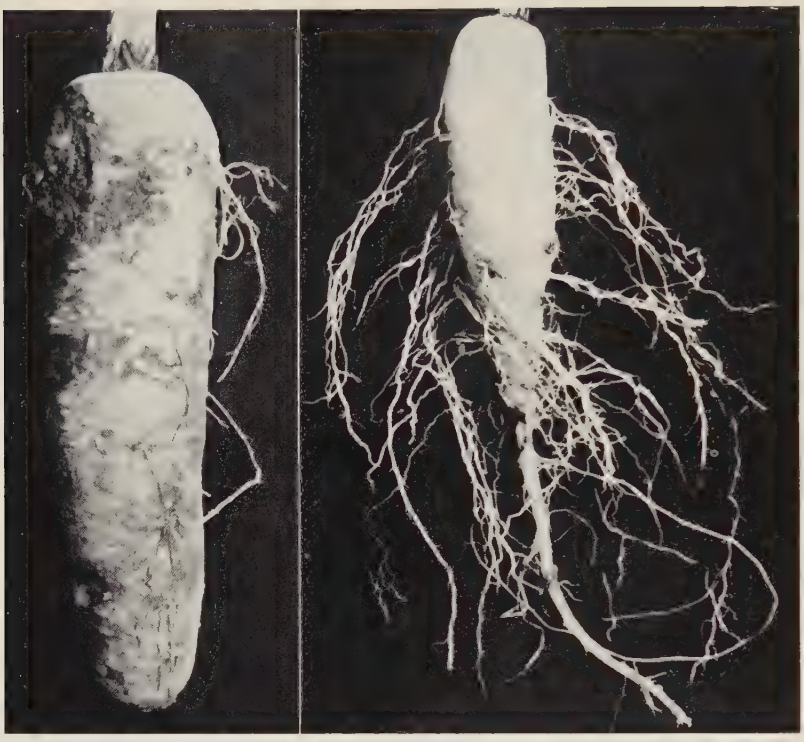

Abb. 1. Rechts: Von *Paratylenchus* befallene, „bärtige" Möhre. Links: Faulstellen an Möhre, verursacht durch primären Befall von Nematoden und sekundär eingedrungenen fäulniserregenden Mikroorganismen.

Abb. 2. Rebenmüdigkeit, verursacht durch Nematoden und Viren.

scheiden zwischen anfälligen Pflanzen, die unter dem Befall stark leiden und Wirtspflanzen, die den Nematoden gute Vermehrungsmöglichkeiten bieten, ohne dass sie stark geschädigt werden (Oostenbrink 1954, 1955, 1956).

Geht man zur Betrachtung des eigentlichen Inhalts der oben skizzierten Ansichten über, so ist zuerst zu fragen, ob es einwandfrei bewiesene Fälle gibt, in denen wandernde Nematoden allein die beobachteten Symptome und Schäden verursacht haben. Diese Frage ist nach dem heutigen Stand unserer Kenntnisse zu bejahen. Es sind zwar nur wenige Arten, aber in einer Reihe eindeutiger Versuche wurde gezeigt, dass z.B. *Pratylenchus penetrans* allein grosse Schäden anrichten kann (Oostenbrink 1955, 1956; Seinhorst, Klinkenberg & v. d. Meer 1956). Ähnliches gilt mit grosser Wahrscheinlichkeit noch für einige andere *Pratylenchus*-Arten sowie für verschiedene Paratylenchen, wenn auch noch nicht allzuviele exakte Versuche veröffentlicht wurden.

Auch die Zahl der genauen Nachweise eines Zusammenwirkens zwischen Nematoden und anderen Organismen ist nicht sehr gross. Slootweg (1956) konnte zeigen, dass verschiedene Pratylenchen, u.a. *P. penetrans* an Blumenzwiebeln zwar Nekrosen verursachen können, dass das Bild der eigentlichen Wurzelfäule aber erst durch die Mitwirkung von Sekundärorganismen, besonders *Cylindrocarpon radicicola* Wollenw., zustande kommt. Klinkenberg (1955) machte die gleiche Feststellung bei der Schwarzwurzelfäule der Erdbeeren. In beiden Fällen sind natürlich die Nematoden der wichtigere Faktor, da die Pilze ohne sie garnicht in das gesunde Pflanzengewebe eindringen können. Ähnliche Verhältnisse haben wir jetzt bei *Paratylenchus* an Möhren gefunden.

Möhren reagieren auf den Befall durch *Paratylenchus* mit einer extrem starken Bildung von Seitenwurzeln (Abb. 1, r.). Dadurch werden die Pflanzen geschwächt und bilden nur kleine Möhrenkörper aus. Weitere Symptome sind in der Regel nicht zu beobachten. Wenn aber jetzt Sekundärorganismen, wie Bakterien und Pilze, dazukommen, treten an den Wurzeln dunkler gefärbte Nekrosen und Faulstellen auf. Die Wurzeln sterben ab, und die Fäulnis greift auf die Möhre selbst über (Abb. 1, l.). Auch hier sind, wie in den oben erwähnten Fällen, die Nematoden das Hauptagens und können allein durchaus grosse Schäden hervorrufen. Die Totalschäden treten aber erst auf, wenn andere Organismen dazukommen. In anderen Fällen scheint die Bedeutung der Sekundärorganismen für das Auftreten der Schäden grösser zu sein.

So haben wir an Erbsen auf ostfriesischen Marschböden Fehlstellen mit schlechtem Wuchs und vorzeitigem Vergilben beobachtet, in denen *Pratylenchus, Hoplolaimus* und *Rotylenchus* in grösserer Zahl gefunden wurden. Wir haben aber festgestellt, dass die Nematoden nur unter bestimmten Bedingungen in der Lage sind, derartige Schäden hervorzurufen, und es scheint, dass dabei das Vorhandensein von Mikroorganismen eine grosse Rolle spielt. In Holland wurde ein ähnlicher Fall auf leichten Böden beobachtet (Labruyère & Seinhorst 1954; Seinhorst 1954). Neuerdings haben wir im Weinbau Beobachtungen gemacht, die auf eine Assoziation von wandernden Wurzelnematoden mit bodenübertragbaren Viren hindeuten. Im westdeutschen Weinbaugebiet findet man in Weinbergen nesterartige Wachstumsdepressionen, die sich langsam ringförmig ausbreiten. Die Reben kümmern und sterben langsam ab (Abb. 2). Neupflanzungen sind zwecklos, da sie ebenfalls nach kurzer Zeit eingehen. In der Rhizosphäre fanden sich hauptsächlich Nematoden der Gattungen *Pratylenchus, Paratylenchus, Criconemoides* und *Rotylenchus*. Die Zusammensetzung der Nematodenfauna schwankt etwas je nach Bodenart. Nach unseren bisherigen Untersuchungen spielt der direkte Angriff der Nematoden nicht die ausschlaggebende Rolle. Sie scheinen vielmehr als Wegbereiter bodenübertragbarer Viren zu fungieren.

Die angeführten Beispiele zeigen, dass wandernde Nematoden in zahlreichen Fällen erst im Zusammenwirken mit anderen Organismen ihre grosse wirtschaftliche Bedeutung erlangen. Es kann also sowohl die erste als auch die zweite der oben angeführten Meinungen durch sichere Untersuchungen belegt werden, was bei *Pratylenchus penetrans* zu einem Widerspruch zu führen scheint. Dieser Widerspruch löst sich aber auf, wenn man bedenkt, dass unter natürlichen Bedingungen nur selten Nematoden allein vorkommen. In allen Fällen, in denen Schäden durch wandernde Wurzelnematoden beobachtet wurden, dürften auch andere Organismen beteiligt sein. Nur der Grad der Beteiligung ist sehr verschieden. In der Praxis werden alle Arten von Zusammenwirken vorkommen, von 90 % Nematodenschaden und 10 % Schaden durch andere Organismen bis zum umgekehrten Verhältnis.

Es bleibt jetzt noch die letzte Ansicht zu diskutieren, nach der die hier besprochenen Nematoden nur als mehr oder weniger zufällige Folgeschädlinge eine Rolle spielen. Diese Meinung ist besonders in Zusammenhang mit der sog. Baumschulmüdigkeit geäussert worden (Schander 1955, 1956, dort weitere Literatur). Danach wird die echte Bodenmüdigkeit durch chemische Substanzen verursacht, die vor

allem bei der Zersetzung abgestorbener Wurzeln entstehen und das Wachstum neuer Wurzeln hemmen. Diese Bodenmüdigkeit ist spezifisch, tritt also nur nach Selbstanbau auf. Derartige Fälle wurden aus Deutschland und Holland (Oostenbrink 1956) berichtet. In beiden Fällen traten die Müdigkeitserscheinungen weitgehend unabhängig vom Nematodenbefall auf. Demgegenüber sind einwandfrei von Nematoden verursachte Wachstumshemmungen an Baumschulgewächsen bekannt, die aber nicht so streng spezifisch sind. Es handelt sich offenbar um ganz verschiedene Arten der Bodenmüdigkeit. Weder Giftstoffe noch Nematoden sind die einzige Ursache der Bodenmüdigkeit. Diese ist, wie immer wieder betont werden muss, überhaupt keine eindeutig definierte Krankheit, sondern ein Schadbild, das auf verschiedene Ursachen zurückgehen kann. Jeder einzelne Fall muss für sich untersucht und beurteilt werden.

SUMMARY

Experiments with *Pratylenchus* and *Paratylenchus* on carrots show that these nematodes are true primary parasites which influence plant tissues in a different manner. *Paratylenchus* injures plants mainly mechanically whereas *Pratylenchus* causes both mechanical and chemical injury. Under natural conditions, damage to crops arises from a more or less close association and co-operation between nematodes and other organisms. This was found in investigations of *Paratylenchus* attacking carrots, *Hoplolaïmus* attacking peas and various other nematodes attacking vines.

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DISKUSSION

Fr. Paesler: 1.) Wir haben auch festgestellt, dass Bodenmüdigkeitserscheinungen nicht immer auf Nematodenbefall zurückzuführen sind.

2.) Gibt es „bodengebundene" Viren?

B. Weischer: Der Begriff „bodengebundene" Viren ist umstritten. Man versteht darunter Viren, die im Boden von Wurzel zu Wurzel übertragen werden können.

H. Decker: Kann die starke Seitenwurzelbildung bei Möhren auf die Aktivierung der Wuchsstoffproduktion der Pflanze bzw. auf eine durch die angreifenden Nematoden bedingte Wuchsstoffinfiltration zurückgehen?

B. Weischer: Eine direkte Beeinflussung des Wuchsstoffhaushaltes durch die Nematoden ist kaum anzunehmen. Wahrscheinlich ist die verstärkte Seitenwurzelbildung eine Reaktion der Pflanze auf die durch den Nematodenbefall gestörte Wasser- und Nährstoffaufnahme.

Nematologica II, Suppl. (1957) : 413-423 S — E. J. Brill, Leiden

A CRITICAL REVIEW OF CURRENT TECHNIQUES FOR THE STUDY OF MIGRATORY ROOT NEMATODES AS ETIOLOGICAL AGENTS

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A critical review must, however objective the presentation, be to some extent coloured by the viewpoint of the reviewer. This paper is presented from the etiologist's point of view, rather than that of the advisory officer or commercial grower. In these circumstances the large gaps in our present knowledge make it much easier to be destructive than constructive. I will try, however, to avoid this and instead to state plainly the difficulties which exist in this field, to outline the progress made to date and, finally, to caution against the unquestioning acceptance of claims based on methods which leave too many vital questions unanswered. I think I can best begin by describing a problem on which I have been working for the past few years and explaining the difficulties which have been encountered.

PROBLEMS STUDIED

Although cherry replanting problems and root rot of strawberry have also received attention at East Malling I propose to confine my remarks to a specific case of apple "decline" which has occurred in a 20-year-old plantation of the variety Cox's Orange Pippin. A patch of trees are stunted and suffer from die-back and leaf-yellowing; roots are few and weak and recall the stubby-root condition attributed to *Trichodorus christei* Allen in U.S.A. (Thomason and Sher, 1957). The soil is a freely-drained sandy loam derived from Thanet Sand and not markedly deficient in major nutrients. A similar adjacent area has been the subject of much investigation, especially from the aspect of mineral nutrition, and magnesium and iron deficiences have been diagnosed. Treatment of these and other possible deficiencies, coupled with irrigation, have produced some improvement but have not restored normal growth. Sampling has shown that there are very few nematodes in the stunted roots but that more normal roots on mature and replanted trees contain many *Pratylenchus penetrans* (Cobb) Sher and Allen.

Species of *Pratylenchus* (100-200), *Tylenchorhynchus* (75-150) and *Paratylenchus* (100-300) are present in the soil at the above populations per 200 ml. as determined by Oostenbrink elutriator, and *Trichodorus, Criconemoides, Tylenchus* and *Rotylenchus* are present in very small numbers.

ETIOLOGICAL EXPERIMENTS

Three pot experiments have been carried out, using an indirect approach based on partial soil sterilisation techniques. Such methods were chosen partly because of the lack of a more direct means of attack and partly because it was considered that a method embracing all the plant parasitic nematodes would be preferable for an initial approach.

For the first experiment soil was collected from the area of poor growth and treated in three ways namely, heated to 60° C. for one hour (H), fumigated with a dichloropropane-dichloropropene mixture at a rate equivalent to 600 lbs./acre (N) and untreated (U). For comparative purposes a horticultural potting compost (C) was also used. Treatments C, H and N were supplemented by "R" sub-treatments in which an equal inoculum of apple roots containing *Pratylenchus penetrans* was applied to each test plant; numerically this was equivalent to about 1 per cent of the *Pratylenchus* content of the soil of the U pots. Five replicate pots of each treatment were each planted with a small apple seedling, plunged in field soil, protected from contamination by nematodes and examined after five months. It was accepted that each treatment had its limitations but it is not proposed to discuss these or the detailed results of each treatment at present. It is, however, plain from Fig. 1 that growth in treatment U was inferior to that in all others. As it was considered that treatment H suffered the fewest probable side-effects, attention was concentrated on a comparison of this and treatment U. Mean results, expressed graphically in Fig. 3a, show that in treatment H the elimination of all plant parasitic nematodes was accompanied by an approximately three-fold increase in fresh root weight and a doubling of shoot weight. Roots were also very different in appearance, being healthy, white and filamentous in H but blackened and stunted in U (Fig. 2).

In the second experiment the H and U treatments were repeated, but attempts were made to reduce side-effects by cutting the heating time to a minimum (35 minutes) and adding a small inoculum of fumigated soil which, although nematode free, might contain a different residual microflora from the heated soil. For comparative purposes a

R. S. PITCHER: *Migratory root nematodes*

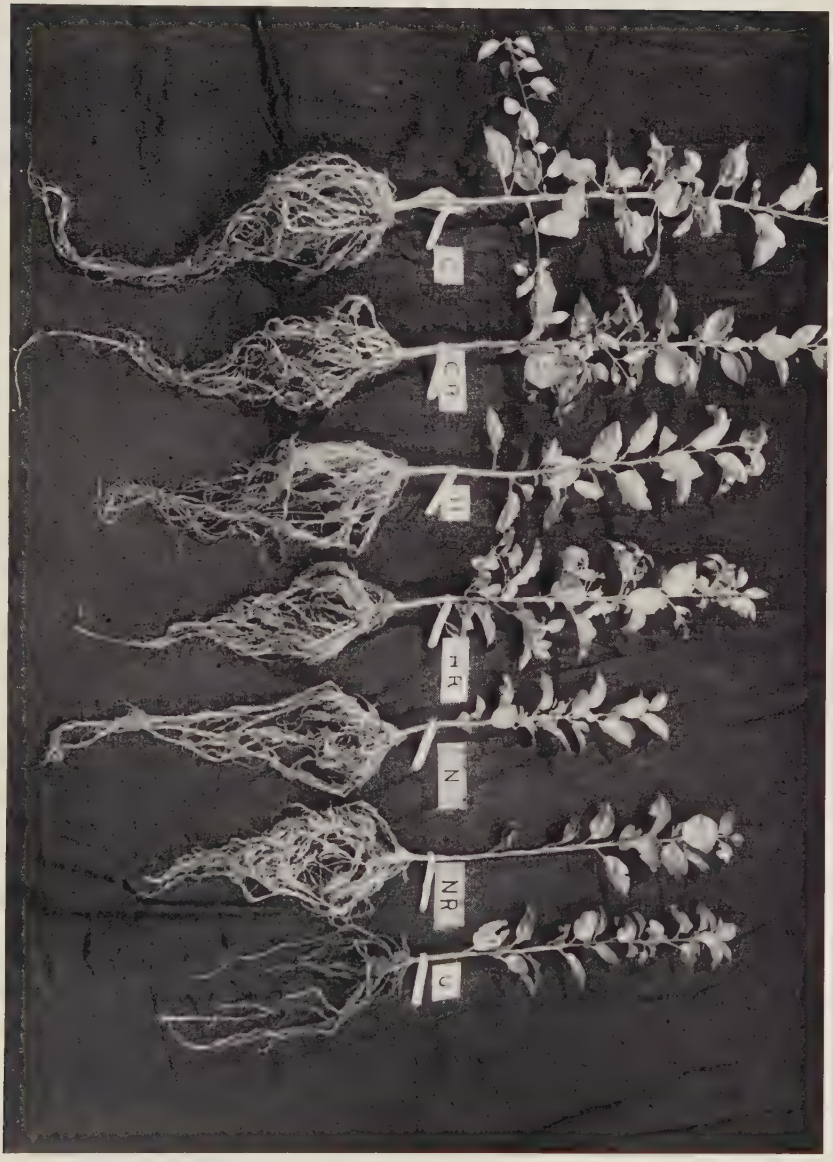

Fig. 1. Growth of apple seedlings in a "decline" soil (U), the same treated with gentle heat (H) or nematicide (N) and in a horticultural compost (C). Sub-treatments R received a light inoculum of *Pratylenchus penetrans*.

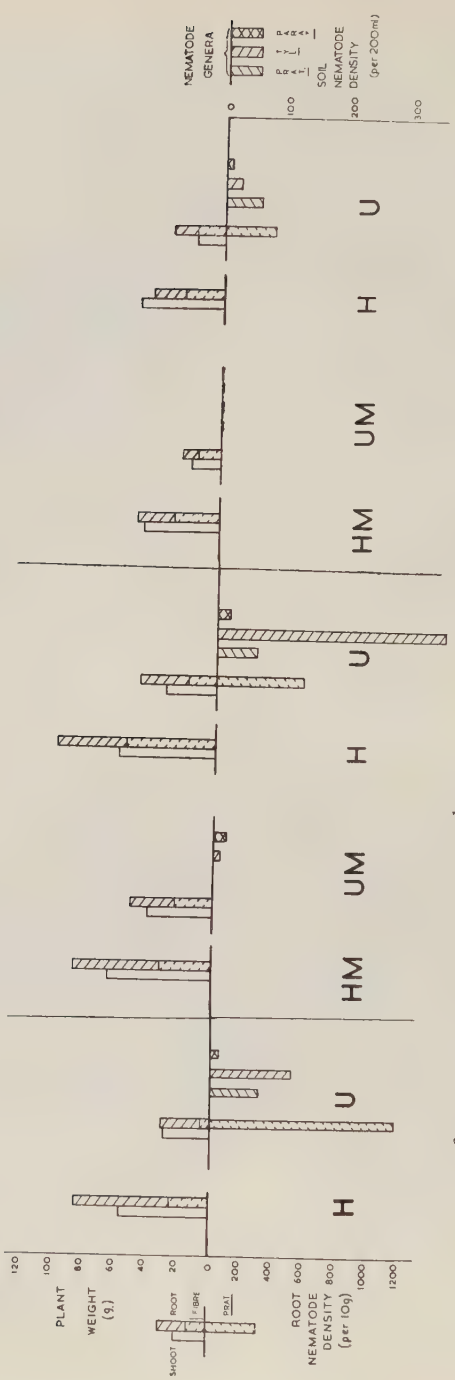

Fig. 3. Effects of gentle heat (H) on nematode population and growth of apple seedlings in two apple orchard soils, a "decline" soil, U and a satisfactory soil, UM.

Fig. 4. Effect of gentle heat (H) on growth of apple seedlings in two apple orchard soils, a "decline" soil U and a satisfactory soil UM.

1) *Soil macro-nutrients*—Analysis showed that treatment H produced no appreciable change in available phosphate or potash but occasionally a small increase in ammonia nitrogen. It is possible, however, that as these samples were taken from pots in which apple seedlings were growing, nitrogen may have been absorbed almost as fast as it was liberated.

2) *Mycorrhizal invasion of roots*—Root samples showed that treatment H greatly inhibited the development of vesicular-arbuscular mycorrhiza.

3) *Heat-labile soil toxins*—Four attempts were made to leach out toxins from heated and untreated soils, two by simple percolation by tap water and two by shaking soil with distilled water for 4-6 hours. In either case nematodes were removed from the leachate by sedimentation and filtration or sieving. The leachate from the percolated soil contained fewer fine soil particles, and probably less soil microflora, than that from a shaken soil. In one experiment leachate from a shaken soil was concentrated about 20 times by freeze-drying. Leachates were either applied to small potted apple seedlings for periods of 6-14 weeks or the radicles of newly germinated apple seedlings were dipped into tubes of leachate for 20-30 days. In no case was there any marked difference between plants treated with H and U leachate. In two experiments plants watered with U leachate were slightly lighter and the roots less healthy than those watered with H leachate, but they were nevertheless much larger and healthier than comparable plants grown in untreated soil. Although these experiments provided no evidence that a heat-labile toxin is responsible for much of the harmful effect of soil U the possibility remains that such a toxin, if present, may not be extractable by the water-leaching techniques used here.

4) *Modification of soil fungistasis*—Dobbs and Hinson (1953) have shown that field soils usually contain a factor which markedly

Table II.

Spore germination of Verticillium albo-atrum in heated (H) and untreated (U) apple "decline" soil and in water.

	Fresh		After 50 Days		
	H	U	H	U	Water
% Spore Germination	70	9	96	0	100

inhibits the germination of spores of a large range of fungi and that such fungistatic effects can be removed by, *inter alia,* heating the soil to 60° C. for one hour. Samples of heated and untreated soil U were compared by a glas-slide/agar technique, using *Verticillium albo-atrum* (Reinke and Berth) as the test species; the results are given in Table 2. From this it is clear that treatment H does destroy a fungistatic factor in soil U and that this effect apparently persists unchanged for at least 50 days. It is impossible to say exactly what the removal of the fungistatic factor means in terms of microfloral balance, breakdown of organic matter and supply of nutrient to the rhizosphere, but the overall effect may well be considerable.

None of these tests define the exact cause of the improved plant growth, but they do, I think, demonstrate side-effects, especially in the microfloral field which give rise to reasonable doubt that the observed effects of heat should be ascribed solely to the removal of nematodes. In my opinion conclusions regarding the plant parasitic status of root migratory nematodes which are based on the results of soil fumigation experiments are even more open to objection. "Soil amendment" effects of fumigants have been noted by other workers (e.g. Peters 1948) and have, for the most part, been interpreted as an inhibition of the nitrifying microflora and a stimulation of the ammonifying (Pochon and Lajudie, 1948; Spencer and Jack, 1950; Tam, 1945 and Thornton, 1952). Discussion of the details of these findings must be left to the microbiologists, but it seems plain that, apart from the obvious phytotoxic risks, most fumigants have subtle side-effects which, on balance, tend to favour plant growth. As these are not fully understood and vary from soil to soil it is quite impossible to predict or evaluate them without a detailed study of each case. I therefore feel grave doubts about accepting the unsupported results of any form of partial soil sterilisation as reliable evidence of nematode injury.

The real problem is to find what part a given nematode species or group of species is playing in a field disease condition in which it is known to be present. There are considerable obstacles in the way of the direct approach to such a situation but it is the logical one to choose if one seeks to prove scientific fact rather than to demonstrate effects whose interpretation must be largely speculative. The difficulties of fulfilling Koch's postulates have been enumerated by Oostenbrink (1956a). These are largely due to the complex environment in which the nematodes live, in which one factor cannot be readily introduced or removed at will. Compliance with the first and simplest of the

Technique	Ref. and details	Advantages	Limitations	Difficulties
ADDITION				
1) Sterile monoculture	Mountain (1955). *Prat.* on excised roots in tissue culture.	Proof of primary pathogenicity. Basis for fundamental studies	Little relation to field conditions. May be difficult to add secondary factors as desired	Laborious and exacting. Small-scale.
2) Unsterile monoculture on whole plants grown in artificial media (including autoclaved soil).	Gadd and Loos (1941) *Prat.* in sand Krusberg and Sasser (1956). *Hopl.* in sand and agar. Holdeman and Graham (1954). *Belon.* in autoclaved soil Birchfield and Martin (1956). *Tyl.* in autoclaved soil.	Permits study of host/parasite relations in more normal conditions than (1). May also be useful as culture method to obtain large nematode inocula for other techniques.	Still remote from field conditions. Difficult to judge real effects of secondary factors.	Some nematodes are hard to establish. Plant growth may be abnormal in autoclaved soil.
3) Unsterile monoculture on whole plants in nematode-free but near-natural media. (including partially sterilised soils with or without checks on microflora)	Thomason and Sher (1957). *Trich.* in 'pasteurised' soil. Schindler (1957). *Xiph.* in 'sterilised' soil. Horn et al (1956). Various in 'sterilised' soil. Raski (1956). *Prat.* in fumigated soil. Oostenbrink (1955) *Prat.* in "sterilised" soil.	As for (2) and approaches field conditions more closely.	Many factors may be modified by soil treatment (e.g. fungistasis), thus altering environment materially.	Not great. Possibly the best compromise as a first step to test nematodes as initiators of plant injury.
4) Unsterile culture with one sp. predominating in nematode-free soils (artificial or natural).	Lownsbery (1956). *Prat.* (+ *Cric.* and *Xiph.*) in fumigated soil.	As for (3). Sometimes side effects can be evaluated by controls	Flora and physical conditions may still be very different from those of the original site. May be side-effects of small populations of other spp.	About as (3).
5) Inoculation with secondary microflora	Pure cultures may be added in any of the above techniques with varying chances of success.			
6) Field populations with one species predominating, induced by selective cropping.	Oostenbrink (1956a and b). *Prat.* spp. and various host plants.	Close approximation to original field conditions. No complicated procedures needed.	Differing side-effects of host plants on soil complexes, and on secondary nematode species.	Not great

Technique	Ref. and details	Advantages	Limitations	Difficulties
REMOVAL				
A) *of Secondary Microflora*				
7) Non-nematicidal fungicides.	Hastings and Bosher (1938). *Prat.* and Malachite green. Slootweg (1956). *Hopl.* and *Prat.* and Thiram.	Nematode injury can be more or less isolated from secondary effects	Cannot exclude all side effects on microflora or on plants.	Not great
B) *of Nematodes*				
8) Mechanical Means.	Slootweg (1956). *Hopl.* and *Prat.* by hammer mill. Seinhorst (1954). *Hop.* and *Prat.* by elutriation and reconstitution.	Flora little affected	Considerable physical effects.	Not great. Needs special apparatus
9) Partial Soil Sterilisation.				
(a) by Heat	Oostenbrink (1954). *Hopl.* and *Prat.* by 60° C. for 2 hrs.	Convenience	Unknown side-effects, chiefly on microflora	Few
(b) by 'selective' nematicides	Various N. America. e.g. Adams (1955) *Xiph.* by BHC Meijneke (1955). *Prat.* by DD. Mountain (1954). *Prat.* by EDB Oostenbrink (1954, 5 and 6). *Prat.* etc. by DD.	"	" " "	"
(c) by combinations of nematicide and fungicide	Various N. America. e.g. Miller (1956). ? sp. EDB and pentachloronitrobenzene on strawberry. Results assessed on root-rot ratings *only*.	"	Unimaginable side-effects	"

postulates is certainly not enough, as non-nematode injuries have more than once been shown to co-exist with root migratory nematodes (Oostenbrink, 1956b; Raski, 1956). The lack of a simple means of culture of root migratory nematodes makes experimental work difficult and the reluctance with which many species establish themselves and multiply under artificial conditions is a further hindrance. It is nevertheless essential, if one intends to discover the real effect of a given nematode on a given plant, to find means of overcoming these difficulties and I have no doubt that, given the time and the will, the task will not prove to be beyond the bounds of human wit.

Even so, success here will tell us only part of the field story, for none of the nematodes act in isolation, but are in balance with a host of other faunal, floral and physical factors. To complete the picture methods must be devised to add or subtract single factors without disturbing the others. This may prove still more formidable than compliance with Koch's postulates, but some modest beginnings have been made and although they may not answer all the queries they are preferable to most of the indirect methods which have been more widely used.

I will conclude with a brief, but world-wide review of techniques (p. 419-20) with notes on the advantages, limitations and difficulties of each. The techniques are classified and divided into two main sections—Addition, comprising nematode inoculation methods and Removal, which includes a variety of more or less selective soil treatments. The classes are arranged in what I suggest to be descending order of reliability of evidence. As in all comparable fields of endeavour, the better the method the higher the quality of the results, but unfortunately shortage of time and manpower may dictate the use of quicker and often less reliable techniques. That this should be so is regrettable, but to draw conclusions far beyond the limitations of the evidence is to be deplored.

ACKNOWLEDGEMENTS

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ZUSAMMENFASSUNG

Die wandernden Wurzelnematoden geben wegen ihrer Verschiedenheit, ihrer Polyphagie und der komplexen Natur ihrer Umgebung dem Ätiologen eine Anzahl spezieller Probleme auf. Aus diesem Grund wurde der schwierige direkte Zugang oft zugunsten einfacher, aber weniger folgerichtiger indirekter Methoden vernachlässigt. Solche Verfahren mögen aus Zeitmangel oder wegen Fehlens geeigneter Hilfskräfte unvermeidbar sein, aber sie sind nur dann gerechtfertigt, wenn die aus ihnen gezogenen Schlussfolgerungen die Grenzen der Gewissheit klar erkennen lassen. Schwierigkeiten, die der Autor beim Studium der die Wurzeln von Obstbäumen angreifenden Wurzelnematoden aufgezählt hat, betonen die Notwendigkeit für eine Verbesserung der Methoden. Versuche an Obstbäumen unter Verwendung partieller Bodensterilisation werden beschrieben und über eine versuchte Analyse der nichtnematiziden Nebenwirkungen langsamer Erhitzung berichtet. Die wesentlichen technischen Verfahren, die bei der Arbeit mit wandernden Wurzelnematoden in allen Teilen der Welt benutzt worden sind, werden unter Berücksichtigung ihrer Vorteile, Grenzen und Schwierigkeiten geprüft.

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DISCUSSION

J. H. Hijner. Perhaps the leachings from heated and unheated pots gave no difference in growth because the toxin, excreted by the nematodes, was heat-stable?

Reply. A heat-stable toxin of this sort may be present and would, as you say, persist in both H and U leachates. It could not, therefore, cause the observed differences in growth, but a heat-labile toxin could do so. The experiments were designed to test for the presence of a heat-labile toxin, probably arising from a plant source, although it could also have come from nematodes.

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TAGETES ALS FEINDPFLANZEN VON *PRATYLENCHUS*-ARTEN

VON

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Vor einigen Jahren berichtete ein praktischer Züchter aus den Niederlanden (v. d. Berg-Smit, 1953), dass er schon 7 Jahre lang günstige Erfahrungen mit Afrikanen, *Tagetes erecta* L., als Vorfrucht von Narzissen in mit Wurzelfäule verseuchtem Boden gemacht habe. Slootweg (1956) zeigte, dass die Krankheit von *Pratylenchus penetrans* (Cobb) Sher et Allen verursacht wird. Er machte es wahrscheinlich, dass es sich hier um eine Bekämpfung dieser Nematoden handelte, obwohl er die Wirkung von *Tagetes* nicht erklären konnte, da er die Wurzeln dieser Pflanze von den Nematoden befallen fand.

Oostenbrink, s'Jacob und Kuiper (1957) zeigten, das *Tagetes patula* L. und *T. erecta* durch *Pratylenchus penetrans* nicht beschädigt werden, und dass sie in verseuchten Böden eine geringere Zahl von Nematoden in den Wurzeln beherbergen als viele andere Gewächse.

Bei anderen Untersuchungen nach den Wirtspflanzen verschiedener *Pratylenchus*-Arten, sowie bei daran anschliessenden Fruchtwechsel- und Praxisversuchen wurden von den Verfassern in der Regel auch *Tagetes*-Arten herangezogen. Die diesbezüglichen Ergebnisse und Erfahrungen werden hier zusammengefasst. Die Untersuchung von Wurzel- und Bodenproben geschah im Laboratorium des Plantenziektenkundige Dienst nach den dort üblichen Verfahren (siehe s'Jacob & Stemerding, 1956).

ERGEBNISSE

A. Wirtspflanzenforschungen 1955 und 1956

Im Jahre 1955 wurden während einer Untersuchung nach den Wirtspflanzen von 3 verschiedenen *Pratylenchus*-Arten Afrikanen (*Tagetes patula nana flore Harmony*) mit anderen Gewächsen verglichen. Dies geschah in 4 Freilandversuchen, und zwar 2 auf Sandboden und 2 auf humusreichem, abgegrabenen Hochmoorboden.

Auf möglichst gleichmässig verseuchten Parzellen wurden im Früh-

jahr 1955 mehrere Reihen mit den Gewächsen besät oder bepflanzt. Nach etwa 3 Monaten wurde von jedem Gewächs eine Wurzelprobe genommen, in den meisten Fällen von 2 Reihen, und auf Nematoden untersucht. Später im Sommer wurden aus den Reihen Afrikanen (*Tagetes patula* L.), Hafer (*Avena sativa* L.), Lupinen (*Lupinus luteus* L.) und von einem Streifen brachliegender Erde überdies eine Bodenprobe genommen und untersucht. Die Proben wurden dem Boden in der Nähe der Wurzeln entnommen, nachdem die oberirdischen Pflanzenteile entfernt waren.

Die Ergebnisse der Wurzeluntersuchungen sind in Tabelle I zusammengefasst. Wie aus der Tabelle hervorgeht, enthielten die Wurzeln von *Tagetes patula* sehr wenig *Pratylenchus*. In allen Versuchen war diese Zahl niedriger als bei den anderen Gewächsen; sie lag denn auch sehr weit unterhalb des Mittelwertes. Auch die Zahl der in 10 g aufgefundenen saproben Nematoden war bei *Tagetes patula* sehr niedrig, um ein Vielfaches weniger als der Mittelwert aller untersuchter Gewächse. Zugleich wurde beobachtet, dass die Wurzeln von *Tagetes* auf allen Parzellen zur Zeit der Probeentnahme ein auffällig helles und gesundes Aussehen zeigten, ohne merkliche Schäden, während bei mehreren anderen Gewächsen die Wurzeln deutlich beschädigt waren; sie waren stellenweise oder ganz braun und faul. Die Zahlen der saproben Nematoden und die niedrigen Zahlen der zu anderen Gattungen

Tabelle I

Routinemässig bestimmte Zahl von Pratylenchus pro 10 g Wurzeln bei Afrikanen, Tagetes patula, und einigen anderen Gewächsen. Probe-Entnahme ungefähr drei Monate nach der Einsaat oder Pflanzung in verseuchte Böden.

Feldversuche 1955 / Gewächse	Ellecom *P. pratensis* 39 Gewächse	Overloon *P. penetrans* 41 Gewächse	Winschoten *P. penetrans* + *P. pratensis* 71 Gewächse	Winschoten *Pratylenchus* sp. 52 Gewächse
Mittel von allen angebauten Gewächsen	7.488	6.055	6.174	1.271
Tagetes patula	234	78	23	23
Avena sativa	5.825	3.175	13.400	2.900
Lupinus luteus	12.325	19.704	30.600	1.350
Tagetes patula in % vom Mittel	3,1 %	1,3 %	0,4 %	1,8 %

gehörenden Tylenchiden, die während der Wurzeluntersuchung ebenfalls gefunden wurden, sind in Tabelle I nicht erwähnt.

Die Bodenprobenuntersuchungen zeigten eindeutig, dass in allen Versuchen die betreffenden *Pratylenchus*-Arten, wie auch die *Tylenchorhynchus*-Arten (hier in erster Linie *T. dubius* (Bütschli) Filipjev) durch *Tagetes patula* in Vergleich zu den anderen Gewächsen auch im Boden stark herabgedrückt worden waren. Dies galt nicht für *Rotylenchus robustus* (De Man) Filipjev (syn. *Hoplolaimus uniformis* Thorne) und wahrscheinlich auch nicht für die übrigen hier vertretenen Tylenchiden, deren Anzahl nach *Tagetes* nicht signifikant niedriger war als nach den anderen Gewächsen. Die Herabsetzung der Zahlen von *Pratylenchus* und *Tylenchorhynchus* im Boden ist offenbar durch die Entwicklung des *Tagetes*-Gewächses bedingt, und ist nicht eine Folge der Fäule von Wurzeln oder Laub, da die Bestimmungen angestellt wurden, als die Pflanzen noch in vollem Wachstum waren. Die Zahlen der Bodenprobenuntersuchungen sind hier nicht erwähnt, weil die soeben genannten Schlussfolgerungen in den nachstehenden Untersuchungen noch weiter dokumentiert worden sind.

In der vorhin erwähnten Weise wurden im Jahre 1956 28 Gewächse auf einer Parzelle mit tonigem Boden geprüft, in der sich eine noch unbeschriebene *Pratylenchus*-Art befindet, die sich von den oben genannten Arten unterscheidet. Auch hier war *Tagetes patula* die Pflanze mit der niedrigsten Anzahl *Pratylenchus* pro 10 g Wurzeln, nämlich null, während der Mittelwert aller Gewächse sich auf 5028 belief. Ergänzende Bodenuntersuchungen wurden in diesem Falle nicht vorgenommen.

B. Fruchtwechseluntersuchungen 1956

Im Laufe des Jahres 1956 wurde von 11 verschiedenen Gewächsen und Brache in dreifacher Wiederholung der Einfluss auf die Populationsdichte von, hauptsächlich, *Pratylenchus pratensis* (de Man) Filipjev bestimmt. Eines dieser Gewächse war *Tagetes patula*. Es wurde am 19. Mai gesät; im folgenden Winter, nachdem die Pflanzen erfroren und ausgetrocknet waren, wurde das Laub entfernt. Im Frühjahr 1957 wurde von jedem Feldchen eine Bodenprobe genommen und untersucht.

Aus Tabelle II geht hervor, dass *Tagetes patula* auch hier die Bevölkerungsdichte von *Pratylenchus* im Vergleich zu anderen Gewächsen und Brache deutlich verringert hat. Auch die Population von *Tylenchorhynchus* (vornehmlich *T. dubius*) ist herabgedrückt worden, die

von *Rotylenchus* (namentlich *R. robustus*) und der übrigen Tylenchiden dagegen nicht. Die Zahl der saproben Nematoden im Boden ist nach *Tagetes patula* nicht niedriger als nach anderen Gewächsen. Dieselben Erfahrungen wie im vorgenannten Versuch wurden im Jahre 1956 in anderen Fruchtwechselversuchen gemacht, in denen die vorherrschende *Pratylenchus*-Art *penetrans* war (siehe Oostenbrink, 1957).

Tabelle II

Nematodenpopulation pro 100 ml Erde im Frühjahr 1957 nach Tagetes patula und anderen Gewächsen.

Die fettgedruckten Zahlen sind das Mittel von 3 Wiederholungsparzellen.
P. = *Pratylenchus pratensis* T. = *Tylenchorhynchus* R. = *Rotylenchus*
U. = übrige *Tylenchida* S. = saprobe Nematoden.

Gewächse 1956	P.	T.	R.	U.	S.
Tagetes patula	5 **2** 0 0	20 **13** 15 5	**70**	**465**	**1087**
Brache	**67**	**32**	**102**	**340**	**732**
10 andere Gewächse	**30—143**	**20—315**	**35—257**	**313—607**	**765—1343**

Im Jahre 1957 wurden in zwei von diesen Versuchen einige empfindliche Gewächse auf allen Parzellen gesät oder gepflanzt. Es stellte sich heraus, dass ihre Wurzeln nach *Tagetes* deutlich weniger *Pratylenchus* enthielten als nach anderen Gewächsen oder Brache. Auf beiden Versuchsfeldern zeigt sich *Tagetes patula* als eine sehr gute Vorfrucht, in den meisten Fällen sogar als die beste.

C. Tagetes patula zwischen anderen Pflanzen oder in der Nähe derselben

Im Jahre 1956 wurden auf einer Parzelle Ackerland mit gemischter Verseuchung von *Pratylenchus pratensis* und *P. penetrans* 6 Paare veredelte Apfelbäumchen (Golden Delicious auf Type E.M. IV) gepflanzt. Um den Fuss des einen Bäumchens von jedem Paar herum wurde eine Oberfläche von 4 Quadratmetern mit *Tagetes patula* in Reihen mit 25 Zentimeter Zwischenraum gesät, 1½ g Saat pro Quadratmeter entsprechend. Als das Gewächs zu Beginn des Frühjahrs 1957 erfroren war, wurde es untergegraben. Um die anderen Bäumchen herum wurde die Erde schwarz belassen. Im Frühjahre 1957 wurde in der Nähe jeden Bäumchens eine Bodenprobe genommen. Bei den meisten Tylenchiden und den saproben Nematoden wurden

keine deutlichen Unterschiede festgestellt, aber die Zahlen von *Pratylenchus* waren bei jedem Bäumchenpaar durch den Anbau von *Tagetes* stark gedrückt. Je 100 Kubikzentimeter Erde enthielten: ohne *Tagetes* 110 — 200 — 185 — 215 — 210 — 160, durchschnittlich 180; mit *Tagetes* 15 — 15 — 20 — 10 — 25 — 35, durchschnittlich 20 *Pratylenchus*.

Im Jahre 1957 wurde um dieselben Bäumchen, wie im Vorjahre, wiederum *Tagetes patula* angebaut; die Erde um die übrigen Bäumchen wurde, wie vorher, schwarz belassen. Im Laufe des Sommers wurde festgestellt, dass die Triebe der mit *Tagetes* umgebenen Bäumchen besser wuchsen.

Auf einer anderen Parzelle, die schwer mit *P. penetrans* verseucht war, stellte sich heraus, dass Apfelbäumchen mit *Tagetes*-Unterwuchs eine sehr viel bessere Vorfrucht für *Rosa laxa* darstellten als Apfelbäumchen allein (siehe Abb. 1).

In einem städtischen Rosarium wurden im Jahre 1956 die Beete wechselweise zur Gesundung mit Unterwuchs von *Tagetes patula* versehen. Im Jahre 1957 waren alle Beete wiederum mit Rosen bepflanzt. Er war auffallend, dass die Entwicklung dieser Pflanzen auf allen Beeten, auf denen *Tagetes* gewachsen war, sehr gut war, auf allen anderen dagegen sehr schlecht. Eine Prüfung im August 1957 ergab, dass die Rosen in den letztgenannten Beeten durch *P. penetrans* befallen waren.

D. Einfluss von kurzfristiger Zucht von Tagetes patula

Im Jahre 1956 wurde *Tagetes patula* als Vorfrucht für Kartoffeln angebaut. Sie wurde am 27. April gesät, lief am 8. Mai auf und wurde am 1. Juni entfernt. Auf drei Parzellen von je 4 Quadratmetern wurden die *Tagetes*-Planzen gänzlich untergegraben, auf drei nur das Laub, auf drei nur die Wurzeln, während drei weitere Parzellen nicht mit *Tagetes* besät waren. Am 1. Juni wurden vorgekeimte Kartoffeln (Noorderling) gepflanzt, die am 25. September gerodet wurden. Aus den Nematoden- und Ernteanalysen ging hervor, dass der Anbau von *Tagetes* während 35 Tagen die *Pratylenchus*-Bevölkerung im Boden und in den Wurzeln der Kartoffeln kaum oder nicht herabgedrückt hatte, und dass dieses Gewächs keinen merklichen Einfluss auf die Entwicklung der Pflanzen gehabt hatte.

Auf einem anderen Acker, der mit *Pratylenchus pratensis* verseucht war, wurde im Jahre 1956 der Einfluss von *Tagetes patula* als Nachernte zu Kartoffeln untersucht. Es wurden verglichen: *Tagetes*

Abb. 1. *Rosa laxa* 1957. Im Vordergrund mit 5 *Pratylenchus penetrans* pro 10 g Wurzeln nach Apfelbäumchen mit *Tagetes patula* als Unterwuchs im Jahre 1956. Im Hintergrund mit 1820 *Pratylenchus* pro 10 g Wurzeln nach Apfelbäumchen ohne Unterwuchs. Die *Tagetes* wurde am 26.4.'56 in Reihen gesät, 1,5 g Saat pro m^2 entsprechend; am 24.8.'56 wurden die oberirdischen Teile der Pflanzen entfernt.

patula als Hauptfrucht, *Solanum tuberosum* mit *Tagetes patula* als Nachfrucht, *Solanum tuberosum* und 5 weitere Gewächse. Alle Pflanzen standen in Wiederholung auf Parzellen von etwa 10 Quadratmetern. Aus Tabelle III geht hervor, dass die *Pratylenchus*-Popu-

Tabelle III

Pratylenchus pratensis-Population in der Erde nach Tagetes patula, Solanum tuberosum, S. tuberosum + T. patula als Herbstgewächs und einigen anderen Gewächsen.

Fettgedruckte Zahlen sind Mittel von 2 Wiederholungsparzellen.

Gewächse 1956	*Pratylenchus* pro 100 ml Erde 1956/'57	
T. patula als Hauptgewächs	**30**	30 30
S. tuberosum + T. patula als Herbstgewächs	**173**	190 155
S. tuberosum	**320**	220 420
5 andere Gewächse	**180—253**	

lation durch *Tagetes* als Nachfrucht deutlich weniger herabgedrückt wurde als durch *Tagetes* als Hauptfrucht. Für *Tylenchorhynchus*, dessen Zahlen hier nicht erwähnt sind, galt dasselbe, während *Rotylenchus* und die saproben Nematoden wiederum unbeeinflusst blieben.

Tabelle IV

Periodisch bestimmte Pratylenchus-Dichte in den Wurzeln von Rosa canina und Tagetes patula und in der umgebenden Erde im Jahre 1956 in einem mit Pratylenchus penetrans + P. pratensis verseuchten Felde.

Datum der Untersuchung	12/6	13/7	15/8	18/9	18/10	30/11	8/1/'57
Pro 100 ml Erde:							
Rosa canina	375	125	170	115	100	85	190
Tagetes patula	318	240	50	0	15	0	25
Pro 10 g Wurzeln:							
Rosa canina	4500	5400	4013	1720	3630	2575	1110
Tagetes patula	200	60	90	0	5	0	0

Um den Zeitpunkt zu ermitteln, an dem *Tagetes* die *Pratylenchus*-Population beeinflusst, wurde auf einer verseuchten Parzelle die Populationsdichte von *Pratylenchus* monatlich sowohl in den Wurzeln von *Tagetes* (gesät am 19.5.1956) wie im Boden dazwischen und auf vergleichbaren Feldchen mit *Rosa canina* (gesät am 4.7.1956) bestimmt.

Aus Tabelle IV geht hervor, dass *Tagetes* erst am 15. August einen merklichen, am 18. September einen sehr deutlichen Einfluss auf die Populationshöhe von *Pratylenchus* hatte. Die Wirkung dieses Gewächses ist also erst nach 3 bis 4 Monaten offenbar geworden.

E. Vergleich verschiedener Rassen von Tagetes patula und T. erecta

Im Jahre 1956 wurden 8 Rassen von *Tagetes patula* und 8 von *T. erecta* zum Vergleich mit anderen Gewächsen auf einem Acker mit *Pratylenchus penetrans* und einem Acker mit hauptsächlich *P. pratensis* angebaut. Die *Tagetes*-Feldchen lagen zwischen den mit anderen Kulturen bestellten Parzellen.

Es zeigte sich, dass alle Rassen von *Tagetes* auf dem erstgenannten Felde deutlich weniger *Pratylenchus* in den Wurzeln hatten als jedes

Tabelle V

Vergleich verschiedener Varietäten von Tagetes patula und T. erecta mit anderen Gewächsen.

	Versuch I, mit *Pratylenchus penetrans*		Versuch II, mit *Pratylenchus pratensis*			
Gewächse 1956	*Pratylenchus* pro 10 g Wurzeln im September 1956		*Pratylenchus* pro 100 ml Erde 1956/'57		*Pratylenchus* pro 10 g Wurzeln von *Secale cereale* im Frühjahr 1957	
Tagetes patula nana flore						
simplice Red head	43		30		30	
simplice Flash	50		35		95	
simplice Sunny	15		50		113	
simplice Naughty Marietta	50		20		173	
pleno Monarch strain mixed	15		18		50	
pleno Tangerine	20		30		123	
pleno Harmony	0		25		75	
pleno Golden Ball	0	**24**	18	**28**	48	**88**
Tagetes patula flora pleno						
Burpee's primrose						
Colorado sunshine	5		20		38	
Sunset giant mixed	45		25		198	
Goldilocks	70		23		153	
Yellow supreme	268		35		68	
Burpee's gold (geruchlos)	130		35		185	
Smiles	105		20		75	
All double orange	30	**82**	23	**23**	83	**100**
Brache				**167**		**795**
Solanum tuberosum				**220**		**1495**
Secale cereale				**175**		**1350**
20 andere Gewächse	**483—21750**					

der anderen Gewächse. Auf dem zweiten Felde war der Zustand ähnlich, wie aus dem Verseuchungsgrad des Bodens nach diesen Gewächsen und aus dem Befall der im nächsten Jahre angebauten Kontrollpflanze Roggen hervorging (siehe Tabelle V).

Aus diesen Experimenten dürfen wir die Schlussfolgerung ziehen, dass alle untersuchten *Tagetes*-Rassen die Populationen von *Pratylenchus penetrans* und von *P. pratensis* stark herabdrücken.

DISKUSSION UND SCHLUSSFOLGERUNGEN

Aus den soeben angeführten Tatsachen geht hervor, dass der Anbau von *Tagetes patula* als Hauptfrucht verseuchte Böden von *Pratylenchus*-Arten säubert, und zwar viel intensiver als andere untersuchte Gewächse und als Brache. In den meisten Fällen wird der Verseuchungsgrad um mehr als 90 % gedrückt, das heisst, ihre Wirkung kommt der einer erfolgreichen Bodenentseuchung mit Nematiziden nahe. Sie wirkt offenbar in 3 verschiedenen Bodenarten (Sand, leichter Ton, und durch Torfmoorabgrabung freigelegter, humusreicher Boden) und gegen 4 verschiedene *Pratylenchus*-Arten (*P. penetrans, P. pratensis* und 2 weitere Arten). Auch *Tylenchorhynchus dubius* wird allem Anschein nach herabgedrückt; *Rotylenchus robustus*, eine Anzahl weiterer Tylenchiden und die saproben Nematoden dagegen werden nicht beeinflusst.

Auch beim Anbau von *Tagetes* zwischen anderen Gewächsen oder rings um sie herum wird die *Pratylenchus*-Bevölkerung in den Wurzeln dieser Gewächsen sowie im benachbarten Boden herabgedrückt. Wenn es sich hierbei um Dauerpflanzen handelt, die stehen bleiben, wie Obstbäumchen oder Ziersträucher, kann ihr Wachstum sichtbar gefördert werden (siehe Seite 427). Nachdem sie gerodet sind, können andere Gewächse in den folgenden Jahren davon Nutzen ziehen (Abb. 1). Der Anbau von *Tagetes* auf mit *Pratylenchus* verseuchtem Boden um neugepflanzte Obstbäume oder Ziersträucher oder zur Genesung schlechtwachsender Bäume oder Sträucher bietet also vielleicht praktische Möglichkeiten. In einigen Versuchen stellte sich aber heraus, dass die Aussaat von *Tagetes* vor oder zugleich mit anderen Gewächsen auch Wachstumshemmungen verursachen kann. Diese Erscheinung dürfte darauf zurückzuführen sein, dass dem Boden dann zuviel Feuchtigkeit entzogen wurde.

Beim Anbau von *Tagetes* im Frühling oder im Herbst während eines kurzen Zeitraumes wurde keine oder eine nur sehr geringe Herabsetzung der *Pratylenchus*-Zahlen beobachtet (siehe Seite 427 u. Tabelle

III). Wenn *Tagetes* im Frühling gesät wird, sind für eine gute Wirkung 3-4 Monate erforderlich (Tabelle IV). Dieselbe Erfahrung hat, nach einer persönlichen Mitteilung, auch SLOOTWEG gemacht.

Aus früheren Untersuchungen wurde schon geschlossen, dass *Tagetes patula* und *T. erecta* nicht durch *Pratylenchus penetrans* beschädigt werden und sehr wenig Älchen in den Wurzeln enthalten. Dies gilt bezüglich *T. patula* auch sicher für die anderen untersuchten *Pratylenchus*-Arten. Alle geprüften Rassen von *Tagetes patula* und *T. erecta* haben mit Rücksicht auf *Pratylenchus penetrans* und *P. pratensis* dieselbe gute Wirkung, die offenbar nicht vom Untergraben des Laubes oder von der Verwesung der Wurzeln abhängig ist. *Tagetes* wirkt auch nicht als Fangpflanze, wie *Crotalaria spectabilis* bei *Meloidogyne* sp. und die resistenten *Solanum andigenum*-Rassen gegen *Heterodera rostochiensis* (auct. div.). Da die *Pratylenchus*-Populationen bis weit unterhalb der Höhe herabgedrückt werden, die durch Brachliegen erreicht wird, handelt es sich bei *Tagetes* also um einen aktiven Mechanismus gegen die Älchen. Der Umstand, dass schon vom Anfang an die Älchen stärker von *Tagetes*-Wurzeln abgewehrt werden als von den Wurzeln anderer Gewächse, lässt vermuten, dass *Tagetes* direkt nematizid wirkt [1]). Diese Wirkung ist spezifischer als jene der Nematizide Dichlorpropen, Aethylendibromid u.a., da sie zwar auch *Tylenchorhynchus*-Arten betrifft, *Rotylenchus robustus* und viele übrigen Tylenchiden jedoch nicht. Ein älteres Literatur-Verzeichnis meldet auch eine Wirkung gegen eine *Meloidogyne*-Art, obwohl *Criconemoides mutabile* Taylor sich als ein spezialisierter Parasit von *Tagetes* zeigte. Vielleicht werden besonders die endoparasitisch lebenden Arten getroffen, und nicht die Ektoparasiten, wie *R. robustus* und *C. mutabile*, oder Arten, die *Tagetes* nicht befallen.

Die Anwendung von *Tagetes* zur Säuberung von Böden, die mit bestimmten Nematoden-Arten verseucht sind, bietet also für die Praxis vielleicht gewisse Möglichkeiten. Die Saatgutgewinnung verdient in diesem Verband Interesse. Es wird versucht, bei der pharmazeutischen Industrie für die Verarbeitungsmöglichkeit einer *Tagetes*-Ernte Interesse zu wecken und auch das Prinzip der nematiziden Wirkung wird bereits untersucht. Auch abgesehen von dem direkten praktischen Wert ist im Hinblick auf Resistenzzüchtung und Fruchtwechselforschung

1) Nach UHLENBROEK & BIJLOO (Vortrag Sekt. VII, Internationaler Pflanzenschutz-Kongress Hamburg, 1957) hat u.a. der von *Tagetes* bekannte Stoff α-Terthienyl in der Tat eine stark nematizide Wirkung.

die Einsicht in den Mechanismus dieses auffälligen Einflusses von *Tagetes* von Bedeutung.

SUMMARY

An old record by STEINER (1941) and an observation by V. D. BERG-SMIT (1953), confirmed and extended by SLOOTWEG (1956), led the authors to study the relationship between *Tagetes* and a number of root-infesting nematodes.

Tagetes patula as a main crop appears to suppress *Pratylenchus* populations in the soil. About 90 % reduction is normally obtained compared with other crops and with fallow. This effect does not depend on decay of haulms or roots in the soil, but is apparently due to nematicidal action of the growing *Tagetes* plants (p. 425, tables 2 and 3). The roots of *Tagetes* harbour fewer *Pratylenchus* specimens during the whole growing season than any other plant tested (tables 1 and 4). The effect is observed in different soil types (sand, peat and silt) and against different *Pratylenchus* species (*P. penetrans, P. pratensis* and two other species). *Tylenchorhynchus dubius* is also suppressed but *Rotylenchus robustus* (syn. *Hoplolaimus uniformis*), a number of other *Tylenchida* and saprozoic nematodes are not affected.

Sixteen varieties of *Tagetes patula* and *T. erecta* were effective (table 5). A growing period of 3-4 months was necessary. Cultivation of *Tagetes* for a shorter period in spring or in the autumn was not effective (p. 428, tables 3 and 4), Cultivation of *Tagetes* between the rows or round the foot of other plants suppressed the *Pratylenchus* population in the roots of the plants as well as in the soil. This resulted in better growth of perennials in the second year or in better growth of the succeeding crop (fig. 1). Exceptionally a temporary adverse influence was noticed, probably connected with the competition for water or some other unfavourable influence on the physical condition of the soil by the *Tagetes* crop.

The nematicidal action of *Tagetes* is more specific than the effect of a soil treatment with known nematicides. Certain species are suppressed, but others are not and some ectoparasitic species (*Rotylenchus robustus, Criconemoides mutabile*) seem to breed without difficulty on *Tagetes* roots. This specificity cannot be fully explained.

Tagetes may perhaps prove useful in the alleviation of attacks on perennials, the prevention of damage to new plantings and in other special cases. Production of cheap *Tagetes* seed and a profitable market for the *Tagetes* crop would help to make their cultivation an economic method of nematode control.

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NEMATOLOGISCHE UNTERSUCHUNGEN AN HOPFEN

VON

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Als in dem Bayerischen Hopfenanbaugebiet Hallertau die Welkekrankheit des Hopfens zunächst sporadisch und im vergangenen Jahr erstmalig in grösseren Umfange auftrat, wurden Sachverständige verschiedener Abteilungen des Pflanzenschutzes zu Rate gezogen. Dabei wurde auch die Meinung laut, es könnte sich zumindest in einigen Fällen um Nematodenschäden handeln.

Bestimmend für diese Meinung war, dass fast immer der Befall fleckenweise auftrat und meist in der Bearbeitungsrichtung in die Länge gezogen erschien. Dazu kam, dass verschiedentlich keine typische Verticilliose nachgewiesen werden konnte.

In diesem Sommer begannen in grösserem Umfange Untersuchungen über alle mit der Welkekrankheit zusammenhängenden Fragen. Der Verfasser konnte dabei nematologisch arbeiten, mit dem Ziel, Zusammenhänge zwischen Pilz und Nematoden zu finden. Da wir jedoch bis jetzt noch keinen deutlichen Zusammenhang zwischen *Verticillium alboatrum* RKE. & Berth. (zweifellos der Ursache des eigentlichen Krankheitsbildes) und Nematoden erkennen, soll in den folgenden Ausführungen nur das Methodisch-Technische gesagt und einige Bemerkungen über den Besatz der Hopfengärten mit Nematoden gemacht werden. Das bis jetzt vorliegende Material muss erst ausgewertet werden und das konnte während der Vegetationsperiode noch nicht geschehen.

METHODIK

Als Vorbemerkung zum Methodischen sei gesagt, dass alle Apparate möglichst Exkursionsgeräte sein mussten, da ja vielfach ohne regelrechtes Labor gearbeitet wird und ausserdem auch Nematodenuntersuchungen an Ort und Stelle nötig sind und durchgeführt werden müssen.

Cystenbildende Nematoden

Da die FENWICK-Kanne als stationäres Gerät nicht in Frage kam, wurde die einfachere Methode mit zwei Sieben gewählt. Da aber andererseits die Untersuchung möglichst rasch vor sich gehen muss, sollte Untersuchungsschale und Auswaschgerät möglichst in einem Stück gefertigt sein. Das Ergebnis zeigt das Bild (Abb. 1). Das Gerät besteht aus drei Teilen: Einen Topf von 35 cm Durchmesser und 30 cm Höhe mit Deckel (auf dem Bild rechts), einem Sieb von ½ mm Maschen-

Abb. 1. Apparat zum Auswaschen und Untersuchen von Erdproben mit cystenbildenden Nematoden. (phot. ZISCH/Wolnzach).

weite in einer weiss emaillierten Fassung von 25 cm Höhe (Mitte) und einem Sieb von 2 mm Maschenweite in einfacher Messingfassung (links). Letzteres Sieb wird beim Auswaschen auf den schrägen Rand des feineren Siebes gestellt und die lufttrockene Erde von oben mit einem gewöhnlichen Gummischlauch hindurchgespült. Nachdem dies geschehen ist und die durchgespülte Erde kräftig ausgewaschen wurde, kommt das feine Sieb in den Topf, der bis etwa 23 cm mit Wasser gefüllt ist. Alle leichten Bestandteile werden dabei nach oben gewirbelt und können von dem schrägen, weissen Rand mit einer Lupe abgezählt werden, während die schwereren Bodenteilchen unten auf dem Sieb liegen bleiben (Abb. 2). Die Entfernung dieser dunklen Masse von der Wasseroberfläche ist dabei so gross, dass sie bei der Untersuchung nicht stört. Für den feldmässigen Gebrauch werden die beiden Siebe

ineinander gestellt, in den Topf gesetzt und dieser mit dem Deckel verschlossen. So ist das Gerät überall hin leicht zu transportieren und

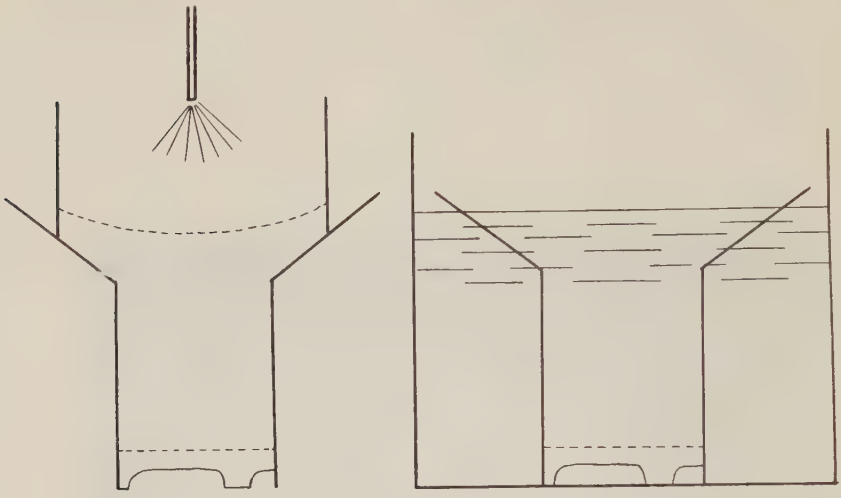

Abb. 2. Schematische Darstellung des Waschapparates für cystenbildende Nematoden. Links beim Auswaschen, rechts beim Untersuchen.

kann an jedem Wasserhahn in Betrieb genommen werden. Mit etwa 50 g Erde arbeitet das Gerät einwandfrei.

Freilebende Nematoden

Hier wurde, da es wieder auf Beweglichkeit und Einfachheit ankam, die Methode von SEINHORST (1955) verwendet. Allerdings gehen bei den im allgemeinen ziemlich schweren Hopfenböden etwa 40-50 % der Gesamtpopulation verloren, doch bleibt das Artenspektrum ziemlich gut erhalten, wie aus vergleichenden Messungen mit verschiedenen anderen Apparaturen hervorging. Die Trichtervorstösse werden mit einem Normalschliff auf die Erlenmeyerkolben gesetzt, so dass sich die Gummimanschette erübrigt. Ausgewaschen werden jeweils 200 g feuchte Erde und die erhaltene Suspension nach einem Absetzen von etwa 12 Stunden (eine Nacht) in hohen Flaschen mit einer Saugpipette auf 40 ccm eingeengt. (Diese 40 ccm haben keine bestimmte Bedeutung, sondern entsprechen den hier verwendeten Tablettenröhrchen.) Zum Auszählen werden dann jeweils 2 ccm nach kräftigem Aufschütteln abpipettiert und in eine Zählkammer gegeben.

Ursprünglich habe ich gewöhnliche Zählkammern mit geritztem

Boden verwendet. Diese waren aber sehr ungünstig, da man vielfach Tiere, die sich in der Zwischenzeit bewegt haben, zweimal zählt. Deshalb wurde die im Bild gezeigte Zählkammer mit voneinander getrennten Zählstreifen entwickelt (Abb. 3). Es handelt sich um einen mit Rinnen versehenen Plexiglasblock von 78 mm Länge, 30 mm Breite und 6 mm Höhe. Die 10 Rinnen haben oben eine Breite von 5 mm und am Boden

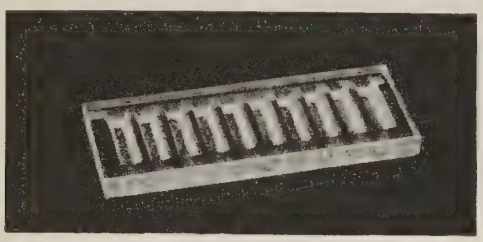

Abb. 3. Zählkammer aus Plexiglas mit voneinander getrennten Zählstreifen. (phot. Zisch/Wolnzach).

von 3 mm bei 5 mm Tiefe. Dadurch ergeben sich schräge Wände, die beim Mikroskopieren nicht verzerren und jedes Objekt auf den Boden sinken lassen. Der Boden jeder Rinne ist poliert, so dass ohne weiteres mit allen Schmutzteilchen, die in einer Probe sind, ausgezählt werden kann. Damit erübrigt sich das Abwandernlassen der Tiere von Baumwollefiltern. Die Zählkammer ist so berechnet, das der Boden der Rinne genau den hellen Gesichtskreis eines Zeiss-Winkel-Mikroskopes mit Objektiv 2,5 ausfüllt (Vergrösserung mit Okular 12,5 32fach linear).

Diese Vergrösserung genügt in den meisten Fällen, um zu erkennen, um welche Gattung es sich handelt. Man kann auch sehr leicht unter dem Mikroskop aus dieser Zählkammer einzelne Tiere zu einer genaueren Bestimmung mit der Pipette herausfangen. Natürlich kann man auch mit der nächststärkeren Vergrösserung (80- oder 125-fach) arbeiten. Allerdings wird dann das Bild etwas lichtschwach und die Verzerrung im Wasser stärker. Die Zählkammer hat sich jedenfalls bis jetzt glänzend bewährt und gestattet ein sehr rasches Auszählen, da man nur jeweils hin oder her zählt, wobei kein Tier verloren gehen kann oder gar zweimal gezählt wird.

Die Probenahme

Bis jetzt konnten wir noch keine ganz einwandfreie Probenahme finden. Im allgemeinen entnehmen wir die Proben in den Hopfengärten von mehreren Stöcken mit einem Esslöffel aus dem Bereich der Sommerwurzeln der Pflanze. Es wird natürlich darauf geachtet,

dass die jeweilige Gesamtprobe immer einen guten Durchschnitt des Hopfengartens darstellt. Es wäre wünschenswert, mit der Zeit zu einer ähnlich einfachen Art der Probenahme zu kommen, wie sie für cystenbildende Arten bereits existiert. Die Proben werden deshalb aus dem obersten Bereich der Pflanze entnommen, weil nur in dieser Region eine Möglichkeit zur Vermehrung von pflanzenparasitären Nematoden gegeben ist. Die Hopfenpflanze wird im Frühjahr aufgedeckt, geschnitten und dann im Laufe des Sommers mehrere Male zugeackert. Im Bereich des alten Wurzelstockes geschieht kaum eine Erdbewegung, dementsprechend ist hier nur eine sehr geringe Saugwurzelbildung. Die für Nematoden günstigen Wurzeln sind die von den Reben im Laufe des Sommers gebildeten Adventivwurzeln. Eine Untersuchung eines Stockes sei hier angeführt:

Tabelle I

Gesamtzahl pflanzenparasitärer Nematoden an einem Hopfenstock in verschiedener Bodentiefe. Die Werte beziehen sich jeweils auf 100 g feuchte Erde.

Tiefe in cm	Nematodenzahl
0-10	210
10-20	250
20-30	130
30-40	60
40-50	10

Unter 50 cm Tiefe trifft man im unmittelbaren Wurzelbereich fast nur *Rhabditis*-Arten, also rein saprobe Formen.

ERGEBNISSE DER BISHERIGEN UNTERSUCHUNGEN

Zunächst sei noch einmal betont, dass die Ergebnisse im einzelnen noch nicht ausgewertet sind und daher nur ganz allgemeine Erkenntnisse vermittelt werden können.

In fast allen Gärten, also kranken wie gesunden, finden sich Vertreter der Gattungen *Tylenchus, Tylenchorhynchus, Psilenchus, Rotylenchus* und *Pratylenchus*. Gelegentlich treten auch *Ditylenchus-*, *Hoplolaimus-* und *Paratylenchus*-Arten auf. In den leichteren Böden erscheinen mehr die schlankeren und kleineren Formen, während die grösseren in den schwereren Böden verhältnismässig häufig sind. Zahlenmässig ist *Pratylenchus* nicht sehr häufig, kann jedoch besonders in kranken Gärten in grösserer Zahl (bis 150 Tiere je 100 g Erde) auftreten. Unter den Dorylaimoideen findet sich gelegentlich *Longidorus*, doch scheint diese Gattung nicht sehr häufig zu sein.

Die Populationsdichte liegt in gesunden Gärten bei 125 (65-170), in welkekranken Gärten bei 340 (100-900) Tiere je 100 g feuchte Erde. Es handelt sich hier um zunächst noch grobe Durchschnittswerte, doch hoffen wir, in absehbarer Zeit genauere Angaben zu erhalten. Die Zahl von 250 Tieren scheint nach unserer gegenwärtigen Kenntnis die kritische Zahl für eine Welkeerkrankung zu sein. Es erscheint dies wenig, doch muss man bedenken, dass bei der verwendeten Methode etwa 40-50 % der Tiere verloren gehen.

Heterodera humuli Filipjev als cystenbildende Art kommt in fast allen Hopfengärten vor. Eine Arbeit, die sich speziell mit dieser Art etwas befassen soll, befindet sich in Vorbereitung. *H. humuli* scheint jedoch nach allen bisher vorliegenden Ergebnissen nicht unmittelbar mit der Welkekrankheit zusammenzuhängen. In einem genau untersuchten Garten liefen in den Versuchsparzellen die Zahlen der erkrankten Stöcke und der Cysten einander genau entgegengesetzt, während die Zahl freilebender parasitärer Nematoden ziemlich der der kranken Stöcke entsprach.

ZUSAMMENSPIEL VON NEMATODEN UND *Verticillium*

Nematoden werden bei einer grösseren Populationsdichte ganz sicher zu Ertragsdepressionen und zu einer Schwächung der Pflanze führen. Dies könnte schon eine Möglichkeit für einen Pilzbefall bilden. Doch weit wichtiger erscheint mir die Saugtätigkeit der Nematoden, weil die Tiere durch die Verletzungen, die sie an den Wurzeln hervorrufen, für Pilze Eintrittsmöglichkeiten in die Pflanze schaffen. Interessant ist, dass der Stock selbst fast nie von *Verticillium* befallen ist, sondern nur die Rebe. Hier geschieht der Befall vom Bereich der Adventivwurzeln her und gerade in diesem Bereich finden sich auch die meisten Nematoden. Aber wie bereits gesagt, sind wir uns über die genauen Zusammenhänge noch nicht klar. Experimentelle Untersuchungen darüber sind bereits im Gange und werden uns hoffentlich bald einigermassen Aufschluss darüber geben.

SUMMARY

During the study of *Verticillium*-wilt on hop in Bavaria (Germany), it was thought that nematodes might be of importance because the damage was always found on distinct patches. As no relation could be found between the numbers of hop root nematodes (i.e. *Heterodera humuli*) and that of diseased plants, migratory parasitic nematodes were investigated.

An apparatus is described (Fig. 1 & 2) for washing and counting cysts, using two sieves. It is easily transportable and can be used wherever a water tap is available.

The apparatus of SEINHORST, using two Erlenmeyer flasks, was used for the migratory nematodes. Counts were made in a special slide (Fig. 3) of plexiglas. Samples were taken at the level in the soil where the hop plant forms its secondary roots. Much deeper down, about 10 in., most nematodes were saprophytic, presumably *Rhabditis* spp.

The genera of plant parasitic nematodes found in most hop plantations are: *Tylenchus, Psilenchus, Tylenchorhynchus, Rotylenchus, Pratylenchus.* Occasionally *Ditylenchus, Hoplolaimus* and *Paratylenchus* occur.

The population density (all non-cyst forming species) was 125 eelworms (65-170) per 100 g. of moist soil in non-diseased hop gardens and about 340 (100-900) in diseased gardens. Sometimes more than 500 cysts of *H. humuli* were found in 100 g. of soil.

The author supposes that root lesions caused by nematode attack provide sites for the entry of the *Verticillium* fungus.

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